ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 November 14; 19(42): 7219-7488

Baishideng Publishing Group
www.wjgnet.com

WJG

World Journal of Gastroenterology

A peer-reviewed, online, open-access journal of gastroenterology and hepatology

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2010-2013

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WJG

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 42 November 14, 2013

EDITORIAL

7219 Understanding and treatment of chronic pancreatitis

Drewes AM

TOPIC HIGHLIGHT

7222 Animal models of pancreatitis: Can it be translated to human pain study?

Zhao JB, Liao DH, Nissen TD

7231 Pathophysiology of chronic pancreatitis

Brock C, Nielsen LM, Lelic D, Drewes AM

7241 Morphological and functional evaluation of chronic pancreatitis with magnetic resonance imaging

Hansen TM, Nilsson M, Gram M, Frøkjær JB

7247 Ultrasonography in diagnosing chronic pancreatitis: New aspects

Dimcevski G, Erchinger FG, Havre R, Gilja OH

7258 Diagnosis and treatment of pancreatic exocrine insufficiency

Lindkvist B

7267 Nutrition in chronic pancreatitis

Rasmussen HH, Irtun Ø, Olesen SS, Drewes AM, Holst M

7276 Diagnosis and treatment of diabetes mellitus in chronic pancreatitis

Ewald N, Hardt PD

7282 Pain and chronic pancreatitis: A complex interplay of multiple mechanisms

Poulsen JL, Olesen SS, Malver LP, Frøkjær JB, Drewes AM

7292 Pharmacological pain management in chronic pancreatitis

Olesen SS, Juel J, Graversen C, Kolesnikov Y, Wilder-Smith OHG, Drewes AM

7302 Pharmacological challenges in chronic pancreatitis

Olesen AE, Brokjaer A, Fischer IW, Larsen IM

7308 Endoscopic management of complications of chronic pancreatitis

Dumonceau JM, Macias-Gomez C

World Journal of Gastroenterology
Volume 19 Number 42 November 14, 2013

REVIEW

7316 Multimodal treatment of hepatocellular carcinoma on cirrhosis: An update
Vivarelli M, Montalti R, Risaliti A

7327 Hepatobiliary manifestations in inflammatory bowel disease: The gut, the drugs and the liver
Rojas-Feria M, Castro M, Suárez E, Ampuero J, Romero-Gómez M

7341 Physiological and molecular biochemical mechanisms of bile formation
Reshetnyak VI

7361 Role of cyclooxygenase-2 in gastric cancer development and progression
Cheng J, Fan XM

MINIREVIEWS

7369 Intestinal acyl-CoA synthetase 5: Activation of long chain fatty acids and behind
Klaus C, Jeon MK, Kaemmerer E, Gassler N

ORIGINAL ARTICLE

7374 1,3-Bis(2-chloroethyl)-1-nitrosourea enhances the inhibitory effect of Resveratrol on 5-fluorouracil sensitive/resistant colon cancer cells
Das D, Preet R, Mohapatra P, Satapathy SR, Kundu CN

7389 Radiofrequency ablation of hepatocellular carcinoma sized > 3 and ⩽ 5 cm: Is ablative margin of more than 1 cm justified?
Ke S, Ding XM, Qian XJ, Zhou YM, Cao BX, Gao K, Sun WB

BRIEF ARTICLE

7399 Interleukin 28B-related polymorphisms: A pathway for understanding hepatitis C virus infection?
Garcia RFL, Moreira S, Ramos ALA, Ferreira LE, Mattos AA, Tovo CV, Nader LA, Ramos JA, Rondinelli E, Dominici AJ, Garcia CE, Pinho MSL, Brandão-Mello CE, Villela-Nogueira CA, França PHC

7405 Short and long-term outcomes of laparoscopic colectomy in obese patients
Vignali A, De Nardi P, Ghirardelli L, Di Palo S, Staudacher C

7412 Is increased red cell distribution width an indicating marker of nonalcoholic steatohepatitis and fibrotic stage?
Cengiz M, Candır BA, Yılmaz G, Akyol G, Ozenirler S

7419 Real-time virtual sonography visualization and its clinical application in biliopancreatic disease
Sofuni A, Itoi T, Itokawa F, Tsuchiya T, Kurihara T, Ishii K, Tsuji S, Ikeuchi N, Tanaka R, Umeda J, Tonozuka R, Honjo M, Mukai S, Moriyasu F

7426 Hemorrhagic gastric and duodenal ulcers after the Great East Japan Earthquake Disaster
Yamanaka K, Miyatani H, Yoshida Y, Asabe S, Yoshida T, Nakano M, Obara S, Endo H

7433 Differentiation between dysplastic nodule and early-stage hepatocellular carcinoma: The utility of conventional MR imaging
Chou CT, Chou JM, Chang TA, Huang SF, Chen CB, Chen YL, Chen RC

7440 Curcumin protects against acetaminophen-induced apoptosis in hepatic injury
Li G, Chen JB, Wang C, Xu Z, Nie H, Qin XY, Chen XM, Gong Q

7447 Prognostic factors in non-malignant and non-cirrhotic patients with portal cavernoma: An 8-year retrospective single-center study
Qi XS, Bai M, He CY, Yin ZX, Guo WG, Niu J, Wu FF, Han GH

7455 Effect of low-dose amitriptyline on globus pharyngeus and its side effects
You LQ, Liu J, Jia L, Jiang SM, Wang GQ

META-ANALYSIS

7461 Combined radiochemotherapy in patients with locally advanced pancreatic cancer: A meta-analysis
Chen Y, Sun XJ, Jiang TH, Mao AW

CASE REPORT

7472 Massive upper gastrointestinal hemorrhage due to invasive hepatocellular carcinoma and hepato-gastric fistula
Sayana H, Yousef O, Clarkston WK

7476 Pseudomembranous colitis associated with a triple therapy for *Helicobacter pylori* eradication
Trifan A, Girleanu I, Cojocariu C, Sfarti C, Singeap AM, Dorobat C, Grigore L, Stanciu C

7480 Successful treatment of multiple hepatocellular adenomas with percutaneous radiofrequency ablation
Ahn SY, Park SY, Kweon YO, Tak WY, Bae HI, Cho SH

LETTERS TO THE EDITOR 7487 Rifaximin and Crohn's disease
Prantera C, Scribano ML

Contents

World Journal of Gastroenterology
Volume 19 Number 42 November 14, 2013

APPENDIX I-VI Instructions to authors

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Editorial Board Member of *World Journal of Gastroenterology*, Seng-Kee Chuah, MD, Gastrointestinal Motility Unit, Division of Gastroenterology, Kaohsiung Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Kaohsiung 833, Taiwan

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World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

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World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2012 Impact Factor: 2.547 (34/74); Total Cites: 19145 (6/74); Current Articles: 944 (1/74); and Eigenfactor® Score: 0.06035 (6/74).

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NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

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Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
November 14, 2013

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Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

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http://www.wjgnet.com/esps/

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7219

World J Gastroenterol 2013 November 14; 19(42): 7219-7221
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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EDITORIAL

Understanding and treatment of chronic pancreatitis

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Author contributions: Drewes AM solely contributed to this paper.
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Received: June 23, 2013 **Revised:** October 23, 2013
Accepted: November 2, 2013
Published online: November 14, 2013

Abstract

Chronic pancreatitis is characterized by an inflammatory process of the pancreas, which is replaced by fibrosis and progressive destruction. The three major clinical features of chronic pancreatitis are pain, maldigestion, and diabetes. Chronic pancreatitis has a profound impact on social life and employment patterns. In the current issue, different topics highlight experimental models of chronic pancreatitis and bridge findings from recent research to bedside. Although the disease is still difficult to treat the current papers represent useful guidelines on how to approach chronic pancreatitis in the clinical settings with the major aim to improve the patient's suffering and quality of life.

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Key words: Chronic pancreatitis; Pathogenesis; Diabetes; Treatment

Core tip: Chronic pancreatitis has a profound impact on social life and employment patterns. In the current issue of *World Journal of Gastroenterology*, different topics highlight experimental models of chronic pancreatitis and bridge findings from recent research to bedside.

Drewes AM. Understanding and treatment of chronic pancreatitis. *World J Gastroenterol* 2013; 19(42): 7219-7221 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7219.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7219

CHRONIC PANCREATITIS

Chronic pancreatitis is characterized by an inflammatory process of the pancreas, which is replaced by fibrosis and progressive destruction. Clinically, the early phase is typically dominated by pain or recurrent episodes of pancreatitis and complications, whereas in the advanced phase symptoms related to exocrine and/or endocrine insufficiency are seen. Hence, the three major clinical features of chronic pancreatitis are pain, maldigestion, and diabetes. The incidence of the disease has been estimated to 2-10/100000 and seems to be increasing[1], but there are major regional differences, and in some countries the disease is much more prevalent. It is also likely that there are many patients with, *e.g.*, abdominal pain, diarrhea and malnutrition - without diagnostic classification - that in reality suffer from chronic pancreatitis. Apart from the illness the economic burden is also of major importance. Chronic pancreatitis has a profound impact on social life and employment patterns[2]. For society the disease in year 2000 accounted for 327000 hospitalizations, 200000 emergency room visits and 532000 physician visits costing 2.5 billion $ in the United States[3]. Even though excess alcohol intake still is a major risk factor, recent data suggest that in some series less than half of the patients have alcoholic pathogenesis and much attention has been paid to "new" entities such as autoimmune pancreatitis[4].

In the current issue of *World Journal of Gastroenterology*, different topics highlight experimental models of chronic pancreatitis and bridge findings from recent research to bedside[5]. The pathogenesis of pancreatitis is reviewed using the recent "MANNHEIM" classification, which may help us in correct diagnostics and subsequent treatment[6]. Diagnosing of chronic pancreatitis has been challenging and imaging techniques are emerging. The main development has been within magnetic resonance imaging and ultrasonography. Two review papers outlined state-of-the-art as well as arising techniques such as diffusion-weighted imaging to indirectly assess the degree of fibrosis[7]. Contrast enhanced ultrasonography and elastography are other examples of new techniques to *e.g.*, differentiate between malignancy and benign lesions[8]. As support for imaging in diagnostics it is of major importance to assess the degree of exocrine and endocrine insufficiency. The so-called "direct or invasive methods" for assessment of exocrine insufficiency such as the Lundh test has been replaced by methods that are more suitable for screening, although imaging methods such as estimation of pancreatic juice flow during secretin stimulation may end up being a major supplement to screening methods. The pros and cons for different methods to unravel exocrine insufficiency are also outlined in the current issue[9]. Treatment of uncomplicated disease is usually conservative, with the major aim to effectively alleviate pain, maldigestion, and diabetes. Malnutrition due to the lack of enzymes results not only in weight loss, but also in specific deficits in vitamins and nutrients that are essential for normal physiological functioning. Malnutrition as complication to chronic pancreatitis is often overlooked and it is of major importance that gastroenterologists are aware of this in the differential diagnosis of patients with weight loss. The paper about nutrition highlights that depletion of nutrients on the one hand and malabsorption (with potential changes in metabolic activity) on the other shall both be considered to avoid severe complications[10]. Another frequent complication is pancreatic pain. The pain is severe and often postprandial resulting in malnutrition despite adequate enzyme replacement therapy. There has been an increasing understanding of the pathogenesis of pain in chronic pancreatitis. Hence, many patients suffering from undiagnosed extrapancreatic causes for the pain should be treated appropriately. Although the pain can be related to strictures and stones in the main pancreatic duct, new research has questioned the importance of the micro-and macrostructural pathological findings. Neurogenic pain due to destruction of the nerves may rather play a role for the pain in the majority of patients - and should be treated accordingly[11]. In the paper about pain treatment there is an update on the current treatment options and it is highly recommended that clinicians are aware of the possibilities for optimal pain relief[12]. Diabetes complicating chronic pancreatitis (type 3c) is a special entity that differs from type 1 and 2 as a variety of hormones apart from insulin are lacking - and the malabsorption also has to be taken into consideration. Despite the importance of this complication few papers have explored the pathogenesis and treatment of type 3c diabetes and this is also updated in the current issue[13]. Finally, there are many challenges when it comes to pharmacological, endoscopical and surgical treatment of chronic pancreatitis. In the paper about pharmacology it is discussed how the influence of complications to the disease is affecting drug absorption and metabolism[14]. Surgical and endoscopic interventions are mainly reserved for complications such as pseudocysts, abscesses, and malignancies. There has been a major development of endoscopy in diagnosing complications and treatment of complications such as endosonographic transmural drainage of pseudocysts and biliary strictures and this is discussed in the paper about endoscopy[15]. Finally, surgery still has a place in treatment of chronic pancreatitis and should be considered in selected cases.

In conclusion important advances have been made in recent years with respect to our understanding of the pathogenesis of chronic pancreatitis. Although the disease is still difficult to treat the current papers represent useful guidelines on how to approach chronic pancreatitis in the clinical settings with the major aim to improve the patient's suffering and quality of life.

REFERENCES

1 **Yadav D**, Timmons L, Benson JT, Dierkhising RA, Chari ST. Incidence, prevalence, and survival of chronic pancreatitis: a population-based study. *Am J Gastroenterol* 2011; **106**: 2192-2199 [PMID: 21946280 DOI: 10.1038/ajg.2011.328]

2 **Gardner TB**, Kennedy AT, Gelrud A, Banks PA, Vege SS, Gordon SR, Lacy BE. Chronic pancreatitis and its effect on employment and health care experience: results of a prospective American multicenter study. *Pancreas* 2010; **39**: 498-501 [PMID: 20118821 DOI: 10.1097/MPA.0b013e3181c5c693]

3 **Lowenfels AB**, Sullivan T, Fiorianti J, Maisonneuve P. The epidemiology and impact of pancreatic diseases in the United States. *Curr Gastroenterol Rep* 2005; **7**: 90-95 [PMID: 15802095 DOI: 10.1007/s11894-005-0045-6]

4 **Coté GA**, Yadav D, Slivka A, Hawes RH, Anderson MA, Burton FR, Brand RE, Banks PA, Lewis MD, Disario JA, Gardner TB, Gelrud A, Amann ST, Baillie J, Money ME, O'Connell M, Whitcomb DC, Sherman S. Alcohol and smoking as risk factors in an epidemiology study of patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2011; **9**: 266-273; quiz e27 [PMID: 21029787 DOI: 10.1016/j.cgh.2010.10.01]

5 **Zhao JB**, Liao DH, Nissen TD. Animal models of pancreatitis: Can it be translated to human pain study? *World J Gastroenterol* 2013; **19**: 7222-7230 [DOI: 10.3748/wjg.v19.i42.7222]

6 **Brock C**, Nielsen LM, Lelic D, Drewes AM. Pathophysiology of chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7231-7240 [DOI: 10.3748/wjg.v19.i42.7231]

7 **Hansen TM**, Nilsson M, Gram M, Frøkjær JB. Morphological and functional evaluation of chronic pancreatitis with magnetic resonance imaging. *World J Gastroenterol* 2013; **19**: 7241-7246 [DOI: 10.3748/wjg.v19.i42.7241]

8 **Dimcevski G**, Erchinger FG, Havre R, Gilja OH. Ultrasonography in diagnosing chronic pancreatitis: New aspects. *World J Gastroenterol* 2013; **19**: 7247-7257 [DOI: 10.3748/wjg.v19.i42.7247]

9 **Lindkvist B**. Diagnosis and treatment of pancreatic exocrine insufficiency. *World J Gastroenterol* 2013; **19**: 7258-7266 [DOI: 10.3748/wjg.v19.i42.7258]

10 **Rasmussen HH**, Irtun Ø, Olesen SS, Drewes AM, Holst M. Nutrition in chronic pancreatitis. *World J Gastroenterol* 2013;

19: 7267-7275 [DOI: 10.3748/wjg.v19.i42.7267]

11 **Poulsen JL**, Olesen SS, Malver LP, Frøkjær JB, Drewes AM. Pain and chronic pancreatitis: A complex interplay of multiple mechanisms. *World J Gastroenterol* 2013; **19**: 7282-7291 [DOI: 10.3748/wjg.v19.i42.7282]

12 **Olesen SS**, Juel J, Graversen C, Kolesnikov Y, Wilder-Smith OHG, Drewes AM. Pharmacological pain management in chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7292-7301 [DOI: 10.3748/wjg.v19.i42.7292]

13 **Ewald N**, Hardt PD. Diagnosis and treatment of diabetes mellitus in chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7276-7281 [DOI: 10.3748/wjg.v19.i42.7276]

14 **Olesen AE**, Brokjær A, Fischer IW, Larsen IM. Pharmacological challenges in chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7302-7307 [DOI: 10.3748/wjg.v19.i42.7302]

15 **Dumonceau JM**, Macias-Gomez C. Endoscopic management of complications of chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7308-7315 [DOI: 10.3748/wjg.v19.i42.7308]

P- Reviewer: Sarr MG **S- Editor**: Wen LL **L- Editor**: A
E- Editor: Zhang DN

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7222

World J Gastroenterol 2013 November 14; 19(42): 7222-7230
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Animal models of pancreatitis: Can it be translated to human pain study?

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Received: June 13, 2013 **Revised:** August 12, 2013
Accepted: August 20, 2013
Published online: November 14, 2013

Abstract

Chronic pancreatitis affects many individuals around the world, and the study of the underlying mechanisms leading to better treatment possibilities are important tasks. Therefore, animal models are needed to illustrate the basic study of pancreatitis. Recently, animal models of acute and chronic pancreatitis have been thoroughly reviewed, but few reviews address the important aspect on the translation of animal studies to human studies. It is well known that pancreatitis is associated with epigastric pain, but the understanding regarding to mechanisms and appropriate treatment of this pain is still unclear. Using animal models to study pancreatitis associated visceral pain is difficult, however, these types of models are a unique way to reveal the mechanisms behind pancreatitis associated visceral pain. In this review, the animal models of acute, chronic and un-common pancreatitis are briefly outlined and animal models related to pancreatitis associated visceral pain are also addressed.

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Key words: Animal model; Pancreatitis; Visceral pain; Mechanism

Core tip: Choosing the right model of pancreatitis is difficult and the scientific rationale needs to be carefully considered. Furthermore, no model of pancreatitis parallels all classical symptoms and the question under investigation is of importance when choosing a model. One of the main symptoms of chronic pancreatitis is visceral pain and in order to improve the pain treatment and obtain more knowledge about the physiology behind the pancreatitis associated visceral pain, animal models of pancreatitis associated visceral pain are needed.

Zhao JB, Liao DH, Nissen TD. Animal models of pancreatitis: Can it be translated to human pain study? *World J Gastroenterol* 2013; 19(42): 7222-7230 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7222.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7222

INTRODUCTION

Pancreatitis represents a common disorder of the gastrointestinal tract. Acute pancreatitis (AP) has an incidence ranged from 4.9 to 35 per 100000 populations[1], whereas chronic pancreatitis (CP) has an incidence from 2.4 to 4.4 per 100000 populations[2]. The etiology of this disease is complex and so far a variety of environmental factors including alcohol abuse, nicotine habits, hereditary factors, efferent duct obstructions, immunological factors and rare metabolic factors have all been described. However, the pathophysiology of AP and CP remains poorly defined[3]. As a result appropriate therapies are still limited, and prognosis has not improved to date, which is mainly due to the lack of a satisfactory animal model of pancreatitis[4,5].

It is well known that pancreatitis is associated with visceral pain, however, the understanding of pain signaling related to pancreatitis is poor[6]. In order to facilitate the development of new pharmaceutical treatments for AP and CP, characterization of the mediators and receptors or ion channels on the sensory nerve terminals and the pathways of the pain signaling are needed. Therefore, in this aspect, the animal models of pancreatitis are needed in parallel in order to explore the mechanism behind pancreatitis associated visceral pain, as this is difficult to study in humans.

In this review, we briefly outline the animal models of acute, chronic and un-common pancreatitis as well as animal models related to pancreatitis associated visceral pain.

ANIMAL MODELS OF ACUTE PANCREATITIS

AP is an inflammatory condition of the pancreas characterized clinically by abdominal pain and elevated levels of pancreatic enzymes in the blood[7]. Other characteristics of AP include edema, acinar cell necrosis, hemorrhage, and severe inflammation of the pancreas. Severe AP may lead to systemic inflammatory response syndrome and multi-organ dysfunction syndrome, which account for the high mortality rates of AP[8,9]. As it is difficult to study AP in the clinic, animal studies are important in order to understand the pathogenesis of AP, however an AP model which is strictly comparable to human AP is still needed. The current animal models of AP have contributed to our knowledge of mechanisms involved in early cellular events, pathogenesis and pathophysiology of AP[10,11]. We have illustrated the summary of existing AP animal models in Table 1[12-59]. Details of different AP animal models including advantages, disadvantages and clinical relevance can be found in a recently published review[4]. From a methodological aspect, selecting the appropriate AP animal model depends on the objectives of each study as different animal models are targeted to different AP features. For developing the effective treatment for AP in the clinic, continued investigation of AP animal models are needed.

ANIMAL MODELS OF CHRONIC PANCREATITIS

A recently published review[5] has described the most frequently used and best established models for CP in animals. The majority of the animal models are rodent models, since mice and rats are easy to handle and there is a steadily increasing number of genetic models obtained by gene deletion or transgenic expression of genetic variants. In the same way for animal models of AP, the models of CP can be classified into noninvasive or nonsurgical models and invasive or surgical models. Table 2 summarizes different animal models of CP[60-100].

Table 1 Different animal models of acute pancreatitis

Methods	Models and examples
Non-invasive	Hormone-induced
	Acute caerulein pancreatitis of rats[12], mice[13], dogs[14], and syrian hamsters[15]
	Trinidadian scorpion toxin induced acute pancreatitis in dogs[16]
	Alcohol-induced: rats[17-19], cats[20] and dogs[21]
	Immune-mediated
	Ovalbumin in rabbit[22]
	Foreign serum in mice[23] and rat[24]
	Spontaneous model of autoimmune acute pancreatitis mice[25]
	Diet-induced: Fed a choline-deficient diet containing ethionine in mice[26]
	Gene knockout: Interleukin (IL)-1 and tumour necrosis factor-α[27], IL-6[28], IL-10[29], chemoattractant cytokine receptor-1[30], neurokinin-1 receptor[31], intercellular adhesion molecule 1 (ICAM-1)[32], metallothionein-1[33], cathepsin B[34], mouse a2-macroglobulin and murinoglobulin[35], complement factor C5a[36], granulocyte-macrophage colony-stimulating factor[37] and phospholipase A2[38]
	L-arginine-induced: Administration of a large dose of *L*-arginine in rats[39,40]
Invasive	Closed duodenal loop (CDL): Dog[41] and rat[42,43]
	Antegrade pancreatic duct perfusion: Cat[44] and rat[45]
	Various compounds infusion into the pancreatic duct: Rat[46] and dog[47]
	Combined intraductal glycodeoxycholic acid with intravenous caerulein: Rat[48]
	Vascular-induced
	Impairment of pancreatic circulation in dogs[49]
	To occlude pancreatic arteries in rats[50]
	Occlusion of pancreatic veins in dogs[51] and in rats[52]
	Complete but reversible ischaemia of the pancreas by occluding different arteries using microvascular clips: Rats[53] and canine[54]
	Duct ligation
	Ligating the distal bile duct at the level of the duodenum[55]
	Combined pancreatic duct ligation with the secretory stimulation, secretin in dogs[56]
	Combining duct ligation with both secretory stimulation and minimal arterial blood[57]
	Duct-ligated opossums models[58]
	Transient obstruction of the sphincter of Oddi (SO) in Australian brush tailed possums[59]

In the non-invasive models, repetitive caerulein injections are amongst the most widely used models. Firstly, caerulein injections are relatively easy to perform and show a high reliability and reproducibility. Secondly, other compounds mediating injury such as lipopolysaccharides or cyclosporin A can easily be added to the design. Thirdly, serial caerulein injections can be performed in transgenic or knockout animals. It is likely that there are dose and frequency dependency for caerulein. The most translational models include repetitive injections of *L*-arginine, which appears to produce CP similar to that in humans[70-72]. In this model, fibrotic tissues are progressively

Table 2 Different animal models of chronic pancreatitis

Methods	Models and examples
Non-invasive	Caerulein-induced
	Serial caerulein injections in mice[60] and rats[61]
	Combination of repetitive caerulein injections with toxins and other agents such as lipopolysaccharides[62], cyclosporin A[63], dibutyltin dichloride[64] and Alcohol[65-67]
	Intraperitoneal caerulein injections are administered in genetically transformed mice such as TRX-1 transgenic mice[68,69]
	Arginine-induced
	A single *L*-arginine injection in rat[70]
	Serial *L*-arginine injections[70-72]
	Alcohol feeding-induced: Lieber-DeCarli formula[73-76]
	Genetic models: Wistar Bonn/Kobori (WBN/Kob) rats[77-79]; R122H transgenic mice[80]; SPINK3-deficient (SPINK3-/-) mice[81]; CFTR-deficient (cftrm1UNC) mice[82] and CFTR(-/-) pigs[83]; Kif3a-deficient mice[84]; PERK-deficient (PERK-/-) mice[85]; Interleukin 1-β transgenic mice[86]
Invasive	Sodium taurocholate-induced: Retrograde infusion of sodium taurocholate (NaTc) into the pancreatic duct system of the rat[87]
	Oleic acid-induced: Retrograde infusion of oleic acid[72,88-91], viscous solution of zein[92], mixture of zein-oleic acid, or viscous solution consisting of zein-oleic acid-linoleic acid[93,94] into rat pancreatic duct
	Congestion of pancreatic fluid flow: Combination of transient stasis of pancreatic juice flow and mild pancreatic duct injury[95]
	Duct ligation model
	Ligation of the common bile duct close to the duodenum pancreatic tissue in dogs[96], mouse[97] and pigs[98]
	Incomplete pancreatic duct ligation in canine[99]
	Occlusion with two different tissue glues in the rat[100]

replaced with adipose tissue. Due to the high impact of alcohol consumption as a risk factor on the pathogenesis in human pancreatic diseases, alcohol has frequently been used to trigger CP in animal models[73,74]. However, it is still being considered whether a model for CP induced by alcohol alone is feasible or satisfactory. The combination of alcohol feeding with caerulein injections exacerbates the course of pancreatitis and consequently increases pancreatic fibrosis and the loss of parenchyma.

Genetic animal models of CP are suitable for different studies. It is well known that activation of trypsinogen is one of the key events in the early phase of pancreatitis, and therefore genetic abnormalities found in the trypsinogen gene and in its inhibitors might be of particular importance of which R122H transgenic mice[80] are a good example. Transgenic expression of the *R122H* mutation of murine trypsin 4 in the pancreas of mice led to progressive fibrosis and chronic inflammation of the pancreas. Repetitive inductions of experimental pancreatitis with supramaximal doses of cerulein resulted in extensive deposition of collagen in periacinar and perilobular spaces of this transgenic animal. However other genetic models might also help us to understand how CP develops[77-79,81,83-86,101].

Invasive animal models can also be used to induce CP. As an example, retrograde infusion of sodium taurocholate (NaTc) into the pancreatic duct[46] or intraductal infusion of NaTc[72] can generate pancreatitis, however the structure of the pancreatic tissue will return to an almost normal state after 14 d. Retrograde infusion of oleic acid[72,88-91], viscous solution of zein[92], a mixture of zein-oleic acid or a viscous solution consisting of zein-oleic acid-linoleic acid[93,94] into rat pancreatic duct will cause severe pancreatic atrophy with irregular fibrosis and fat replacement over a period of 6 mo. However, these models of pancreatitis appear quite distinct from CP in humans. As one factor alone is inadequate to cause persistent pancreatic injury, a combination of transient stasis of pancreatic juice flow and mild pancreatic duct injury is a well established and reliable method to generate CP in animal models[95]. It is well known that pancreatic ductal hypertension contributes to the pathogenesis of CP; therefore animal models can also be generated by complete obstruction of the pancreatic duct[96-98], incomplete pancreatic duct ligation[99] and occlusion with different tissue glues[100]. Yamamoto *et al*[102] developed an animal model with pancreatic ductal hypertension and demonstrated that this plays an important role in the onset and development of CP in rats. However, models for CP based on duct obstruction are not common and there is only a minority of studies examining the morphological and biochemical changes of the pancreas after duct ligation[41,103,104].

ANIMAL MODELS OF UN-COMMON PANCREATITIS

Un-common types of pancreatitis can include autoimmune pancreatitis (AIP), hereditary pancreatitis[105], groove pancreatitis[106], tropical pancreatitis, pancreatitis in ectopic or heterotopic pancreatic tissue, ascaris-induced pancreatitis, pancreatitis in cystic fibrosis, pancreas divisum, annular pancreas, pancreatic cancer manifesting as AP, and duodenal villous adenoma with pancreatitis. With exception of AIP and hereditary pancreatitis, no relevant animal models were found for other un-common pancreatitis. Furthermore, hereditary pancreatitis animal models were mentioned in the genetic animal models of CP above. Therefore only animal models of AIP are briefly introduced in this section.

To date, several animal models of AIP have been described. The first model involves the adoptive transfer of amylase-specific (an antigen mainly located in acinar cells) CD4$^+$ T cells and results in pancreatitis in naive syngenic recipient animals[107]. Notably, the histological lesions of this model mimic the lobulocentric inflammatory reaction in type 1 AIP. A model developed by immunization of neonatally thymectomized mice with CA (an antigen mainly located on the pancreatic epithelium) and later transfer of CD4$^+$ lymphocytes resulted in a duct-centric pattern of pancreatitis resembling type 2 AIP[108]. In another model, NTx-NFS/sld mice spontaneously developed sialoadenitis in which a-fodrin was involved as an autoantigen, as reported in some patients with Sjogren syndrome and AIP[109]. Transforming growth factor-β

(TGFβ) appears to be an important regulatory factor in maintaining immune homeostasis. Loss of TGFβ signalling contributes to AIP in TGFβ dominant negative mutant mice[110].

Recently two animal models for AIP were proposed. The WBN/Kob rat model, associated with congenital decreased peripheral Tregs spontaneously develops sialoadenitis, thyroiditis, sclerosing cholangitis and tubulointerstitial nephritis[111]. Although the target antigens remain unclear, $CD8^+$ cells may be the effector cell in this rat model[112]. Another recently described animal model of AIP is the Treg-deficient NOD mouse[113]. CD28KO mice spontaneously develop AIP that closely resembles the human disease[113]. More recently, Haruta *et al*[114] investigated the possible involvement of chronic, persistent exposure to avirulent bacteria in the pathogenesis of AIP using C57BL/6 mice.

Existing animal models for AIP have several limitations. In most models the disease is induced by adoptive transfer of autoreactive cells and/or antibodies rather than spontaneous development of the disease with identical antigen specificity. The distribution of lesions produced in animal models for AIP is also variable. This may be attributed to the diversity of target antigens, different methods of immune staining and different mouse strains. In addition, typical histopathological findings of AIP (*e.g.*, lymphoplasmacytic infiltration with fibrosis, obliterative phlebitis and GELs) are rarely observed in animal models. Thus, there is a need to develop spontaneous animal models with identical autoantigens and typical histopathological findings for AIP.

VISCERAL PAIN IN ANIMAL MODELS OF CHRONIC PANCREATITIS

One of the main clinical symptoms of CP in humans is pain, occurring either in episodes or as a constant disabling pain[115,116]. Hence, an important goal of treatment for CP is to relieve the pain. The analgesic treatment is often inadequate as the pathophysiology behind CP as well as the mechanisms behind the accompanying pain is not yet fully understood[117]. As described in the previous sections, no single animal model displays all aspects of CP and each of the different models display histological similarities to the human condition to various degrees. In order to improve the pain treatment and obtain more knowledge about the physiology behind CP associated pain, animal models of CP associated pain are needed.

Rat models of CP where pancreatic nociception was investigated, have been established through invasive, non-invasive and spontaneous models[118,119]. In these models pancreatic pain has been shown through both mechanical and thermal stimulation of the abdomen (referred pain[120]) as well as direct electrical stimulation of the pancreas[118,121]. These models had histopathological similarities to the human disease and had progressive fibrosis and inflammation. Furthermore, the models showed correlation between nociceptive behaviour and increased expression of nerve growth factor (NGF) in the pancreas and calcitonin gene-related peptide (CGRP), substance P (SP), proteinase-activated receptor 2 (PAR2), and brain-derived neurotrophic factor (BDNF) in thoracic dorsal root ganglion and spinal cord segments[118,122-124]. Increased expression of NGF, CGRP, SP and BDNF has also been shown in human patients with CP[125-127].

Several animal models have investigated the mechanisms involved in pain accompanying CP. Takamido *et al*[119] reported morphological changes of the nervous system being involved in development of CP pain. This study suggested that elongation of dorsal root ganglia axons and enlargement of intrapancreatic nerve bundles as being a possible mechanism of pain generation in CP. On a supraspinal level, findings have suggested that descending facilitation from the rostral ventromedial medulla plays an important role in persistent pain associated with CP[128]. Furthermore, recent rat experiments have suggested that spinal microglia becomes activated during CP and has an important role in initiating and maintaining chronic pain[129].

TRANSLATION OF PANCREATITIS-ASSOCIATED VISCERAL PAIN STUDY FROM ANIMAL TO HUMAN

It may be difficult to use animal models to study pancreatitis associated visceral pain as pain is a subjective experience. However animal models are needed to explore the molecular mechanisms behind pancreatitis associated visceral pain as this is difficult to study in humans. The molecular mechanisms behind the chronic pain associated with CP are poorly understood, but within recent years, animal experiments have suggested some mechanisms that might be involved. The transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1) have been shown to be contributing factors to pain in CP[122,130,131]. It has been shown that CP is accompanied by an increased level of NGF which caused an up-regulation of TRPV1 expression and sensitivity, resulting in hyperalgesia and allodynia[122,130]. TRPA1 is important in both inflammation and pain in CP and can be sensitized through activation of PAR2[131].

The mechanisms mentioned above could be used as targets for the development of novel therapeutics, aiming at treating the chronic pain accompanying CP. Neutralizing antibodies against neurotransmitters such as BDNF and NGF[124,130] or receptor specific antagonists[122] has proven to reverse the characteristic nociceptive behavioral changes induced by CP in several of the experimental models. Furthermore, inhibition of trypsin or inhibition of microglia activation has also abrogated the pain related behavior seen in response to CP[123,129]. All these different mechanisms of pain treatment in CP models could have a potential as targets for novel pharmacological treatment of the chronic pain associated with CP in human patients. Also established analgesic drugs such as gabapentin, buprenorphine, and morphine have been tested in animal models of CP[118,121,132], and shown to have analgesic effect. However, many of these therapeutic approaches need to be tested in humans, before their true potential analgesic treatment of CP pain in humans can be established. It is known that some of these analgesic mechanisms are species specific and specific to the different models of induced CP.

CONCLUSION

Choosing the right model of pancreatitis is difficult and the scientific rationale needs to be carefully considered. Furthermore, no model of pancreatitis parallels all classical symptoms and the question under investigation is of importance when choosing a model. One of the main symptoms of CP is visceral pain and in order to improve the pain treatment and obtain more knowledge about the physiology behind the pancreatitis associated visceral pain, animal models of pancreatitis associated visceral pain are needed.

REFERENCES

1 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, Dibonaventura MD, Carroll CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-87.e1-3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]

2 **DiMagno MJ**, DiMagno EP. Chronic pancreatitis. *Curr Opin Gastroenterol* 2012; **28**: 523-531 [PMID: 22782018 DOI: 10.1097/MOG.0b013e3283567dea]

3 **Khokhar AS**, Seidner DL. The pathophysiology of pancreatitis. *Nutr Clin Pract* 2004; **19**: 5-15 [PMID: 16215091 DOI: 10.1177/011542650401900105]

4 **Su KH**, Cuthbertson C, Christophi C. Review of experimental animal models of acute pancreatitis. *HPB (Oxford)* 2006; **8**: 264-286 [PMID: 18333137 DOI: 10.1080/13651820500467358]

5 **Aghdassi AA**, Mayerle J, Christochowitz S, Weiss FU, Sendler M, Lerch MM. Animal models for investigating chronic pancreatitis. *Fibrogenesis Tissue Repair* 2011; **4**: 26 [PMID: 22133269 DOI: 10.1186/1755-1536-4-26]

6 **Barreto SG**, Saccone GT. Pancreatic nociception--revisiting the physiology and pathophysiology. *Pancreatology* 2012; **12**: 104-112 [PMID: 22487519 DOI: 10.1016/j.pan.2012.02.010]

7 **Vege SS**. Pathogenesis of acute pancreatitis. Available from: URL: http://www.uptodate.com/contents/pathogenesis-of-acute-pancreatitis#H2 (15-04-2013)

8 **Chan YC**, Leung PS. Acute pancreatitis: animal models and recent advances in basic research. *Pancreas* 2007; **34**: 1-14 [PMID: 17198179 DOI: 10.1097/01.mpa.0000246658.38375.04]

9 **Steer ML**, Saluja A. Experimental acute pancreatitis: studies of the early events that lead to cell injury. In: Go VLW, Gardner JD, Brooks FP, Lebenthal E, DiMagno EP, Scheele GA, editors. The Pancreas, Biology, Pathobiology and Disease. 2nd ed. New York: Raven Press, 1993: 489-499

10 **Fétaud V**, Frossard JL, Farina A, Pastor CM, Bühler L, Dumonceau JM, Hadengue A, Hochstrasser DF, Lescuyer P. Proteomic profiling in an animal model of acute pancreatitis. *Proteomics* 2008; **8**: 3621-3631 [PMID: 18686302 DOI: 10.1002/pmic.200800066]

11 **Pini M**, Rhodes DH, Castellanos KJ, Hall AR, Cabay RJ, Chennuri R, Grady EF, Fantuzzi G. Role of IL-6 in the resolution of pancreatitis in obese mice. *J Leukoc Biol* 2012; **91**: 957-966 [PMID: 22427681 DOI: 10.1189/jlb.1211627]

12 **Watanabe O**, Baccino FM, Steer ML, Meldolesi J. Supramaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cell: early morphological changes during development of experimental pancreatitis. *Am J Physiol* 1984; **246**: G457-G467 [PMID: 6720895]

13 **Niederau C**, Ferrell LD, Grendell JH. Caerulein-induced acute necrotizing pancreatitis in mice: protective effects of proglumide, benzotript, and secretin. *Gastroenterology* 1985; **88**: 1192-1204 [PMID: 2984080]

14 **Renner IG**, Wisner JR. Exogenous secretin ameliorates ceruletide induced acute pancreatitis in the dog. *Dig Dis Sci* 1983; **28**: 946 (abstract)

15 **Adler G**, Kern HF, Scheele GA. Experimental models and concepts in acute pancreatitis. In: Go VWL, Gardner JD, Brooks FP, Lebenthal E, Di Magno EP, Scheele GA, editors. The exocrine pancreas: biology, pathobiology, diseases. New York: Raven, 1986

16 **Pantoja JL**, Renner IG, Abramson SB, Edmondson HA. Production of acute hemorrhagic pancreatitis in the dog using venom of the scorpion, Buthus quinquestriatus. *Dig Dis Sci* 1983; **28**: 429-439 [PMID: 6839906 DOI: 10.1007/BF02430532]

17 **Siech M**, Heinrich P, Letko G. Development of acute pancreatitis in rats after single ethanol administration and induction of a pancreatic juice edema. *Int J Pancreatol* 1991; **8**: 169-175 [PMID: 2033327]

18 **Weber H**, Merkord J, Jonas L, Wagner A, Schröder H, Käding U, Werner A, Dummler W. Oxygen radical generation and acute pancreatitis: effects of dibutyltin dichloride/ethanol and ethanol on rat pancreas. *Pancreas* 1995; **11**: 382-388 [PMID: 8532655]

19 **Werner J**, Laposata M, Fernández-del Castillo C, Saghir M, Iozzo RV, Lewandrowski KB, Warshaw AL. Pancreatic injury in rats induced by fatty acid ethyl ester, a nonoxidative metabolite of alcohol. *Gastroenterology* 1997; **113**: 286-294 [PMID: 9207289 DOI: 10.1016/S0016-5085(97)70106-9]

20 **Wedgwood KR**, Adler G, Kern H, Reber HA. Effects of oral agents on pancreatic duct permeability. A model of acute alcoholic pancreatitis. *Dig Dis Sci* 1986; **31**: 1081-1088 [PMID: 3757722 DOI: 10.1007/BF01300261]

21 **Friedman HS**, Lowery R, Shaughnessy E, Scorza J. The effects of ethanol on pancreatic blood flow in awake and anesthetized dogs. *Proc Soc Exp Biol Med* 1983; **174**: 377-382 [PMID: 6420795]

22 **THAL A**. Studies on pancreatitis. II. Acute pancreatic necrosis produced experimentally by the arthus sensitization reaction. *Surgery* 1955; **37**: 911-917 [PMID: 14373312]

23 **Janigan DT**, Nevalainen TJ, MacAulay MA, Vethamany VG. Foreign serum-induced pancreatitis in mice. I. A new model of acute pancreatitis. *Lab Invest* 1975; **33**: 591-607 [PMID: 1202281]

24 **Nevalainen TJ**. Pancreatic injury caused by intraductal injection of foreign serum in rat. *Virchows Arch B Cell Pathol* 1978; **27**: 89-98 [PMID: 417460]

25 **Kanno H**, Nose M, Itoh J, Taniguchi Y, Kyogoku M. Spontaneous development of pancreatitis in the MRL/Mp strain of mice in autoimmune mechanism. *Clin Exp Immunol* 1992; **89**: 68-73 [PMID: 1352748 DOI: 10.1111/j.1365-2249.1992.tb06879.x]

26 **Lombardi B**, Estes LW, Longnecker DS. Acute hemorrhagic pancreatitis (massive necrosis) with fat necrosis induced in mice by DL-ethionine fed with a choline-deficient diet. *Am J Pathol* 1975; **79**: 465-480 [PMID: 1094837]

27 **Denham W**, Yang J, Fink G, Denham D, Carter G, Ward K, Norman J. Gene targeting demonstrates additive detrimental effects of interleukin 1 and tumor necrosis factor during pancreatitis. *Gastroenterology* 1997; **113**: 1741-1746 [PMID: 9352880 DOI: 10.1053/gast.1997.v113.pm9352880]

28 **Suzuki S**, Miyasaka K, Jimi A, Funakoshi A. Induction of

acute pancreatitis by cerulein in human IL-6 gene transgenic mice. *Pancreas* 2000; **21**: 86-92 [PMID: 10881937 DOI: 10.1097/00006676-200007000-00056]

29 **Gloor B**, Todd KE, Lane JS, Rigberg DA, Reber HA. Mechanism of increased lung injury after acute pancreatitis in IL-10 knockout mice. *J Surg Res* 1998; **80**: 110-114 [PMID: 9790823 DOI: 10.1006/jsre.1997.5289]

30 **Gerard C**, Frossard JL, Bhatia M, Saluja A, Gerard NP, Lu B, Steer M. Targeted disruption of the beta-chemokine receptor CCR1 protects against pancreatitis-associated lung injury. *J Clin Invest* 1997; **100**: 2022-2027 [PMID: 9329966]

31 **Grady EF**, Yoshimi SK, Maa J, Valeroso D, Vartanian RK, Rahim S, Kim EH, Gerard C, Gerard N, Bunnett NW, Kirkwood KS. Substance P mediates inflammatory oedema in acute pancreatitis via activation of the neurokinin-1 receptor in rats and mice. *Br J Pharmacol* 2000; **130**: 505-512 [PMID: 10821777]

32 **Frossard JL**, Saluja A, Bhagat L, Lee HS, Bhatia M, Hofbauer B, Steer ML. The role of intercellular adhesion molecule 1 and neutrophils in acute pancreatitis and pancreatitis-associated lung injury. *Gastroenterology* 1999; **116**: 694-701 [PMID: 10029629]

33 **Fu K**, Tomita T, Sarras MP, De Lisle RC, Andrews GK. Metallothionein protects against cerulein-induced acute pancreatitis: analysis using transgenic mice. *Pancreas* 1998; **17**: 238-246 [PMID: 9788536 DOI: 10.1097/00006676-199810000-00003]

34 **Halangk W**, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenbuerger M, Reinheckel T, Domschke W, Lippert H, Peters C, Deussing J. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J Clin Invest* 2000; **106**: 773-781 [PMID: 10995788 DOI: 10.1172/JCI9411]

35 **Umans L**, Serneels L, Overbergh L, Stas L, Van Leuven F. alpha2-macroglobulin- and murinoglobulin-1- deficient mice. A mouse model for acute pancreatitis. *Am J Pathol* 1999; **155**: 983-993 [PMID: 10487856]

36 **Bhatia M**, Saluja AK, Singh VP, Frossard JL, Lee HS, Bhagat L, Gerard C, Steer ML. Complement factor C5a exerts an anti-inflammatory effect in acute pancreatitis and associated lung injury. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G974-G978 [PMID: 11292607]

37 **Frossard JL**, Saluja AK, Mach N, Lee HS, Bhagat L, Hadenque A, Rubbia-Brandt L, Dranoff G, Steer ML. In vivo evidence for the role of GM-CSF as a mediator in acute pancreatitis-associated lung injury. *Am J Physiol Lung Cell Mol Physiol* 2002; **283**: L541-L548 [PMID: 12169573]

38 **Mizuma K**, Schröder T, Kaarne M, Korpela H, Lempinen M. Serum phospholipase A2 in diet-induced pancreatitis. *Eur Surg Res* 1984; **16**: 156-161 [PMID: 6202520]

39 **Hegyi P**, Rakonczay Z, Sári R, Góg C, Lonovics J, Takács T, Czakó L. L-arginine-induced experimental pancreatitis. *World J Gastroenterol* 2004; **10**: 2003-2009 [PMID: 15237423]

40 **Varga IS**, Matkovics B, Czako L, Hai DQ, Kotorman M, Takacs T, Sasvari M. Oxidative stress changes in L-arginine-induced pancreatitis in rats. *Pancreas* 1997; **14**: 355-359 [PMID: 9163781 DOI: 10.1097/00006676-199705000-00005]

41 **Pfeffer RB**, Stasior O, Hinton JW. The clinical picture of the sequential development of acute hemorrhagic pancreatitis in the dog. *Surg Forum* 1957; **8**: 248-251 [PMID: 13529599]

42 **Nevalainen TJ**, Seppä A. Acute pancreatitis caused by closed duodenal loop in the rat. *Scand J Gastroenterol* 1975; **10**: 521-527 [PMID: 1153948]

43 **Orda R**, Hadas N, Orda S, Wiznitzer T. Experimental acute pancreatitis. Inducement by taurocholate sodium-trypsin injection into a temporarily closed duodenal loop in the rat. *Arch Surg* 1980; **115**: 327-329 [PMID: 6153522 DOI: 10.1001/archsurg.1980.01380030073018]

44 **Widdison AL**, Alvarez C, Reber HA. The low-pressure duct perfusion model of acute pancreatitis. *Eur Surg Res* 1992; **24** Suppl 1: 55-61 [PMID: 1601024 DOI: 10.1159/000129239]

45 **Olazábal A**. Effect of prostaglandins E2 and I2 and of indomethacin on deoxycholic acid-induced damage to the rat bile-pancreatic duct. *Gastroenterology* 1983; **84**: 928-934 [PMID: 6339311]

46 **Aho HJ**, Nevalainen TJ, Aho AJ. Experimental pancreatitis in the rat. Development of pancreatic necrosis, ischemia and edema after intraductal sodium taurocholate injection. *Eur Surg Res* 1983; **15**: 28-36 [PMID: 6840152]

47 **Musa BE**, Nelson AW, Gillette EL, Ferguson HL, Lumb WV. A model to study acute pancreatitis in the dog. *J Surg Res* 1976; **21**: 51-56 [PMID: 933516 DOI: 10.1016/0022-4804(76)90009-3]

48 **Schmidt J**, Rattner DW, Lewandrowski K, Compton CC, Mandavilli U, Knoefel WT, Warshaw AL. A better model of acute pancreatitis for evaluating therapy. *Ann Surg* 1992; **215**: 44-56 [PMID: 1731649 DOI: 10.1097/00000658-199201000-00007]

49 **Barzilai A**, Ryback BJ, Medina JA, Toth L, Dreiling DA. The morphological changes of the pancreas in hypovolemic shock and the effect of pretreatment with steroids. *Int J Pancreatol* 1987; **2**: 23-32 [PMID: 3681033]

50 **Redha F**, Uhlschmid G, Ammann RW, Freiburghaus AU. Injection of microspheres into pancreatic arteries causes acute hemorrhagic pancreatitis in the rat: a new animal model. *Pancreas* 1990; **5**: 188-193 [PMID: 2315295 DOI: 10.1097/00006676-199003000-00011]

51 **Pfeffer RB**, Lazzarini-Robertson A, Safadi D, Mixter G, Secoy CF, Hinton JW. Gradations of pancreatitis, edematous, through hemorrhagic, experimentally produced by controlled injection of microspheres into blood vessels in dogs. *Surgery* 1962; **51**: 764-769 [PMID: 14486114]

52 **Sjövall S**, Holmin T, Evander A, Stenram U. Splenic and gastro-duodenal vein occlusion--influence on the pancreatic gland and on the outcome of experimental pancreatitis. *Int J Pancreatol* 1988; **3**: 143-149 [PMID: 2452221]

53 **Hoffmann TF**, Leiderer R, Waldner H, Arbogast S, Messmer K. Ischemia reperfusion of the pancreas: a new in vivo model for acute pancreatitis in rats. *Res Exp Med (Berl)* 1995; **195**: 125-144 [PMID: 8570908 DOI: 10.1007/BF02576782]

54 **Broe PJ**, Zuidema GD, Cameron JL. The role of ischemia in acute pancreatitis: studies with an isolated perfused canine pancreas. *Surgery* 1982; **91**: 377-382 [PMID: 6175032]

55 **Baxter JN**, Jenkins SA, Day DW, Roberts NB, Cowell DC, Mackie CR, Shields R. Effects of somatostatin and a long-acting somatostatin analogue on the prevention and treatment of experimentally induced acute pancreatitis in the rat. *Br J Surg* 1985; **72**: 382-385 [PMID: 2581647 DOI: 10.1002/bjs.1800720516]

56 **Popper HL**, Necheles H. Edema of the pancreas. *Surg Gynecol Obstet* 1942; **74**: 123

57 **Popper HL**, Necheles H, Russell KC. Transition of pancreatic edema into pancreatic necrosis. *Surg Gynecol Obstet* 1948; **87**: 79-82 [PMID: 18860330]

58 **Senninger N**, Moody FG, Coelho JC, Van Buren DH. The role of biliary obstruction in the pathogenesis of acute pancreatitis in the opossum. *Surgery* 1986; **99**: 688-693 [PMID: 2424109]

59 **Chen JW**, Thomas A, Woods CM, Schloithe AC, Toouli J, Saccone GT. Sphincter of Oddi dysfunction produces acute pancreatitis in the possum. *Gut* 2000; **47**: 539-545 [PMID: 10986215 DOI: 10.1136/gut.47.4.539]

60 **Neuschwander-Tetri BA**, Burton FR, Presti ME, Britton RS, Janney CG, Garvin PR, Brunt EM, Galvin NJ, Poulos JE. Repetitive self-limited acute pancreatitis induces pancreatic fibrogenesis in the mouse. *Dig Dis Sci* 2000; **45**: 665-674 [PMID: 10759232]

61 **Elsässer HP**, Haake T, Grimmig M, Adler G, Kern HF. Repetitive cerulein-induced pancreatitis and pancreatic fibrosis in the rat. *Pancreas* 1992; **7**: 385-390 [PMID: 1594561 DOI: 10.1097/00006676-199205000-00017]

62 **Segersvärd R**, Sylván M, Lempinen M, Larsson J, Permert J. Impact of chronic and acute high-fat feeding on acute experimental pancreatitis complicated by endotoxinaemia. *Scand J Gastroenterol* 2004; **39**: 74-80 [PMID: 14992565 DOI: 10.1080/00365520310007233]

63 **Vaquero E**, Molero X, Tian X, Salas A, Malagelada JR. Myofibroblast proliferation, fibrosis, and defective pancreatic repair induced by cyclosporin in rats. *Gut* 1999; **45**: 269-277 [PMID: 10403741 DOI: 10.1136/gut.45.2.269]

64 **Sparmann G**, Merkord J, Jäschke A, Nizze H, Jonas L, Löhr M, Liebe S, Emmrich J. Pancreatic fibrosis in experimental pancreatitis induced by dibutyltin dichloride. *Gastroenterology* 1997; **112**: 1664-1672 [PMID: 9136846 DOI: 10.1016/S0016-5085(97)70049-0]

65 **Gukovsky I**, Lugea A, Shahsahebi M, Cheng JH, Hong PP, Jung YJ, Deng QG, French BA, Lungo W, French SW, Tsukamoto H, Pandol SJ. A rat model reproducing key pathological responses of alcoholic chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G68-G79 [PMID: 17884979 DOI: 10.1152/ajpgi.00006.2007]

66 **Deng X**, Wang L, Elm MS, Gabazadeh D, Diorio GJ, Eagon PK, Whitcomb DC. Chronic alcohol consumption accelerates fibrosis in response to cerulein-induced pancreatitis in rats. *Am J Pathol* 2005; **166**: 93-106 [PMID: 15632003 DOI: 10.1016/S0002-9440(10)62235-3]

67 **Perides G**, Tao X, West N, Sharma A, Steer ML. A mouse model of ethanol dependent pancreatic fibrosis. *Gut* 2005; **54**: 1461-1467 [PMID: 15870229 DOI: 10.1136/gut.2004.062919]

68 **Ohashi S**, Nishio A, Nakamura H, Asada M, Tamaki H, Kawasaki K, Fukui T, Yodoi J, Chiba T. Overexpression of redox-active protein thioredoxin-1 prevents development of chronic pancreatitis in mice. *Antioxid Redox Signal* 2006; **8**: 1835-1845 [PMID: 16987036 DOI: 10.1089/ars.2006.8.1835]

69 **Leung PS**, Chan YC. Role of oxidative stress in pancreatic inflammation. *Antioxid Redox Signal* 2009; **11**: 135-165 [PMID: 18837654 DOI: 10.1089/ars.2008.2109]

70 **Delaney CP**, McGeeney KF, Dervan P, Fitzpatrick JM. Pancreatic atrophy: a new model using serial intra-peritoneal injections of L-arginine. *Scand J Gastroenterol* 1993; **28**: 1086-1090 [PMID: 8303212 DOI: 10.3109/00365529309098314]

71 **Weaver C**, Bishop AE, Polak JM. Pancreatic changes elicited by chronic administration of excess L-arginine. *Exp Mol Pathol* 1994; **60**: 71-87 [PMID: 8070543 DOI: 10.1006/exmp.1994.1007]

72 **Yamaguchi T**, Kihara Y, Taguchi M, Nagashio Y, Tashiro M, Nakamura H, Otsuki M. Persistent destruction of the basement membrane of the pancreatic duct contributes to progressive acinar atrophy in rats with experimentally induced pancreatitis. *Pancreas* 2005; **31**: 365-372 [PMID: 16258372 DOI: 10.1097/01.mpa.0000179729.61457.e5]

73 **Lieber CS**, DeCarli LM. Alcoholic liver injury: experimental models in rats and baboons. *Adv Exp Med Biol* 1975; **59**: 379-393 [PMID: 1237225]

74 **Lieber CS**, Decarli LM. Animal models of ethanol dependence and liver injury in rats and baboons. *Fed Proc* 1976; **35**: 1232-1236 [PMID: 944146]

75 **Sarles H**, Lebreuil G, Tasso F, Figarella C, Clemente F, Devaux MA, Fagonde B, Payan H. A comparison of alcoholic pancreatitis in rat and man. *Gut* 1971; **12**: 377-388 [PMID: 4329553 DOI: 10.1136/gut.12.5.377]

76 **Tsukamoto H**, Towner SJ, Yu GS, French SW. Potentiation of ethanol-induced pancreatic injury by dietary fat. Induction of chronic pancreatitis by alcohol in rats. *Am J Pathol* 1988; **131**: 246-257 [PMID: 3358454]

77 **Ohashi K**, Kim JH, Hara H, Aso R, Akimoto T, Nakama K. WBN/Kob rats. A new spontaneously occurring model of chronic pancreatitis. *Int J Pancreatol* 1990; **6**: 231-247 [PMID: 1698893]

78 **Hashimoto T**, Yamada T, Yokoi T, Sano H, Ando H, Nakazawa T, Ohara H, Nomura T, Joh T, Itoh M. Apoptosis of acinar cells is involved in chronic pancreatitis in Wbn/Kob rats: role of glucocorticoids. *Pancreas* 2000; **21**: 296-304 [PMID: 11039475 DOI: 10.1097/00006676-200010000-00012]

79 **Mori M**, Fu X, Chen L, Zhang G, Higuchi K. Hereditary pancreatitis model WBN/Kob rat strain has a unique haplotype in the Pdwk1 region on chromosome 7. *Exp Anim* 2009; **58**: 409-413 [PMID: 19654439 DOI: 10.1538/expanim.58.409]

80 **Archer H**, Jura N, Keller J, Jacobson M, Bar-Sagi D. A mouse model of hereditary pancreatitis generated by transgenic expression of R122H trypsinogen. *Gastroenterology* 2006; **131**: 1844-1855 [PMID: 17087933 DOI: 10.1053/j.gastro.2006.09.049]

81 **Ohmuraya M**, Hirota M, Araki K, Baba H, Yamamura K. Enhanced trypsin activity in pancreatic acinar cells deficient for serine protease inhibitor kazal type 3. *Pancreas* 2006; **33**: 104-106 [PMID: 16804421 DOI: 10.1097/01.mpa.0000226889.86322.9b]

82 **Dimagno MJ**, Lee SH, Hao Y, Zhou SY, McKenna BJ, Owyang C. A proinflammatory, antiapoptotic phenotype underlies the susceptibility to acute pancreatitis in cystic fibrosis transmembrane regulator (-/-) mice. *Gastroenterology* 2005; **129**: 665-681 [PMID: 16083720 DOI: 10.1016/j.gastro.2005.05.059]

83 **Rogers CS**, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, Taft PJ, Rogan MP, Pezzulo AA, Karp PH, Itani OA, Kabel AC, Wohlford-Lenane CL, Davis GJ, Hanfland RA, Smith TL, Samuel M, Wax D, Murphy CN, Rieke A, Whitworth K, Uc A, Starner TD, Brogden KA, Shilyansky J, McCray PB, Zabner J, Prather RS, Welsh MJ. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science* 2008; **321**: 1837-1841 [PMID: 18818360 DOI: 10.1126/science.1163600]

84 **Lin F**, Hiesberger T, Cordes K, Sinclair AM, Goldstein LS, Somlo S, Igarashi P. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc Natl Acad Sci USA* 2003; **100**: 5286-5291 [PMID: 12672950 DOI: 10.1073/pnas.0836980100]

85 **Harding HP**, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, Sabatini DD, Ron D. Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival. *Mol Cell* 2001; **7**: 1153-1163 [PMID: 11430819 DOI: 10.1016/S1097-2765(01)00264-7]

86 **Marrache F**, Tu SP, Bhagat G, Pendyala S, Osterreicher CH, Gordon S, Ramanathan V, Penz-Osterreicher M, Betz KS, Song Z, Wang TC. Overexpression of interleukin-1beta in the murine pancreas results in chronic pancreatitis. *Gastroenterology* 2008; **135**: 1277-1287 [PMID: 18789941 DOI: 10.1053/j.gastro.2008.06.078]

87 **Aho HJ**, Nevalainen TJ, Lindberg RL, Aho AJ. Experimental pancreatitis in the rat. The role of phospholipase A in sodium taurocholate-induced acute haemorrhagic pancreatitis. *Scand J Gastroenterol* 1980; **15**: 1027-1031 [PMID: 6165066 DOI: 10.3109/00365528009181808]

88 **Mundlos S**, Adler G, Schaar M, Koop I, Arnold R. Exocrine pancreatic function in oleic acid-induced pancreatic insufficiency in rats. *Pancreas* 1986; **1**: 29-36 [PMID: 2437560 DOI: 10.1097/00006676-198601000-00007]

89 **Henry JP**, Steinberg WM. Pancreatic function tests in the rat model of chronic pancreatic insufficiency. *Pancreas* 1993; **8**: 622-626 [PMID: 8302800 DOI: 10.1097/00006676-199309000-00015]

90 **Yamaguchi T**, Nakamura H, Kihara Y, Taguchi M, Yoshikawa H, Otsuki M. Long-term overexpression of membrane type-1 matrix metalloproteinase and matrix metalloproteinase-2 in oleic acid-induced pancreatitis in rats. *Pancreas* 2002; **24**: 348-356 [PMID: 11961487 DOI: 10.1097/00006676-200205000-00005]

91 **Tashiro M**, Nakamura H, Taguchi M, Yamaguchi T, Yoshi-

kawa H, Fukumitsu K, Kihara Y, Otsuki M. Oleic acid-induced pancreatitis alters expression of transforming growth factor-beta1 and extracellular matrix components in rats. *Pancreas* 2003; **26**: 197-204 [PMID: 12604920 DOI: 10.1097/00006676-200303000-00018]

92 **Tomita T**, Rhodes J, Falscroft J, Doull V, Kimmel JR, Pollock HG. Endocrine pancreas in the rat model of exocrine pancreatic insufficiency. *Pancreas* 1988; **3**: 568-575 [PMID: 3054870 DOI: 10.1097/00006676-198810000-00010]

93 **Steinberg WM**, Burns MK, Henry JP, Nochomovitz LE, Anderson KK. Cerulein induces hyperplasia of the pancreas in a rat model of chronic pancreatic insufficiency. *Pancreas* 1987; **2**: 176-180 [PMID: 3628223 DOI: 10.1097/00006676-198703000-00009]

94 **Kataoka K**, Sasaki T, Yorizumi H, Sakagami J, Kashima K. Pathophysiologic studies of experimental chronic pancreatitis in rats induced by injection of zein-oleic acid-linoleic acid solution into the pancreatic duct. *Pancreas* 1998; **16**: 289-299 [PMID: 9548669 DOI: 10.1097/00006676-199804000-00014]

95 **Yamaguchi T**, Kihara Y, Yamamoto M, Otsuki M. Transient stasis of pancreatic fluid flow together with mild injury of the pancreatic duct cause chronic pancreatitis. *Dig Dis Sci* 2011; **56**: 2326-2332 [PMID: 21274624 DOI: 10.1007/s10620-011-1586-1]

96 **Churg A**, Richter WR. Early changes in the exocrine pancreas of the dog and rat after ligation of the pancreatic duct. A light and electron microscopic study. *Am J Pathol* 1971; **63**: 521-546 [PMID: 5581235]

97 **Watanabe S**, Abe K, Anbo Y, Katoh H. Changes in the mouse exocrine pancreas after pancreatic duct ligation: a qualitative and quantitative histological study. *Arch Histol Cytol* 1995; **58**: 365-374 [PMID: 8527243 DOI: 10.1679/aohc.58.365]

98 **Boerma D**, Straatsburg IH, Offerhaus GJ, Gouma DJ, van Gulik TM. Experimental model of obstructive, chronic pancreatitis in pigs. *Dig Surg* 2003; **20**: 520-526 [PMID: 14534374 DOI: 10.1159/000073688]

99 **Tanaka T**, Ichiba Y, Fujii Y, Itoh H, Kodama O, Dohi K. New canine model of chronic pancreatitis due to chronic ischemia with incomplete pancreatic duct obstruction. *Digestion* 1988; **41**: 149-155 [PMID: 3224767 DOI: 10.1159/000199767]

100 **Isaksson G**, Lundquist I, Ihse I. Effects on the exocrine and endocrine pancreas of duct occlusion with two different tissue glues in the rat. *Eur Surg Res* 1983; **15**: 136-144 [PMID: 6345169 DOI: 10.1159/000128345]

101 **Cano DA**, Sekine S, Hebrok M. Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis. *Gastroenterology* 2006; **131**: 1856-1869 [PMID: 17123526 DOI: 10.1053/j.gastro.2006.10.050]

102 **Yamamoto M**, Otani M, Otsuki M. A new model of chronic pancreatitis in rats. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G700-G708 [PMID: 16959955 DOI: 10.1152/ajpgi.00502.2005]

103 **Klein ES**, Grateron H, Rudick J, Dreiling DA. Pancreatic intraductal pressure: I. A consideration of regulatory factors. *Am J Gastroenterol* 1983; **78**: 507-509 [PMID: 6881116]

104 **De Rai P**, Franciosi C, Confalonieri GM, Biffi R, Andreoni B, Uggeri F, Malesci A. Effects of somatostatin on acute pancreatitis induced in rats by injection of taurocholate and trypsin into a temporarily closed duodenal loop. *Int J Pancreatol* 1988; **3**: 367-373 [PMID: 2902146]

105 **Rosendahl J**, Bödeker H, Mössner J, Teich N. Hereditary chronic pancreatitis. *Orphanet J Rare Dis* 2007; **2**: 1 [PMID: 17204147 DOI: 10.1186/1750-1172-2-1]

106 **Tyagi P**, Thaper S, Bhatia V, Sharma M. Often a missed type of pancreatitis: groove pancreatitis. *Indian J Gastroenterol* 2012; **31**: 215-216 [PMID: 22829067 DOI: 10.1007/s12664-012-0229-6]

107 **Zen Y**, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, Nakanuma Y. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007; **45**: 1538-1546 [PMID: 17518371 DOI: 10.1002/hep.21697]

108 **Okazaki K**, Uchida K, Ohana M, Nakase H, Uose S, Inai M, Matsushima Y, Katamura K, Ohmori K, Chiba T. Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. *Gastroenterology* 2000; **118**: 573-581 [PMID: 10702209 DOI: 10.1016/S0016-5085(00)70264-2]

109 **Haneji N**, Nakamura T, Takio K, Yanagi K, Higashiyama H, Saito I, Noji S, Sugino H, Hayashi Y. Identification of alpha-fodrin as a candidate autoantigen in primary Sjögren's syndrome. *Science* 1997; **276**: 604-607 [PMID: 9110981]

110 **Hahm KB**, Im YH, Lee C, Parks WT, Bang YJ, Green JE, Kim SJ. Loss of TGF-beta signaling contributes to autoimmune pancreatitis. *J Clin Invest* 2000; **105**: 1057-1065 [PMID: 10772650 DOI: 10.1172/JCI8337]

111 **Sakaguchi Y**, Inaba M, Tsuda M, Quan GK, Omae M, Ando Y, Uchida K, Okazaki K, Ikehara S. The Wistar Bonn Kobori rat, a unique animal model for autoimmune pancreatitis with extrapancreatic exocrinopathy. *Clin Exp Immunol* 2008; **152**: 1-12 [PMID: 18279444 DOI: 10.1111/j.1365-2249.2008.03588.x]

112 **Kountouras J**, Zavos C, Chatzopoulos D. A concept on the role of Helicobacter pylori infection in autoimmune pancreatitis. *J Cell Mol Med* 2005; **9**: 196-207 [PMID: 15784177 DOI: 10.1111/j.1582-4934.2005.tb00349.x]

113 **Meagher C**, Tang Q, Fife BT, Bour-Jordan H, Wu J, Pardoux C, Bi M, Melli K, Bluestone JA. Spontaneous development of a pancreatic exocrine disease in CD28-deficient NOD mice. *J Immunol* 2008; **180**: 7793-7803 [PMID: 18523243]

114 **Haruta I**, Yanagisawa N, Kawamura S, Furukawa T, Shimizu K, Kato H, Kobayashi M, Shiratori K, Yagi J. A mouse model of autoimmune pancreatitis with salivary gland involvement triggered by innate immunity via persistent exposure to avirulent bacteria. *Lab Invest* 2010; **90**: 1757-1769 [PMID: 20733561 DOI: 10.1038/labinvest.2010.153]

115 **Ammann RW**. Diagnosis and management of chronic pancreatitis: current knowledge. *Swiss Med Wkly* 2006; **136**: 166-174 [PMID: 16633964]

116 **Andrén-Sandberg A**, Hoem D, Gislason H. Pain management in chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2002; **14**: 957-970 [PMID: 12352215 DOI: 10.1097/00042737-200209000-00006]

117 **DiMagno EP**. Toward understanding (and management) of painful chronic pancreatitis. *Gastroenterology* 1999; **116**: 1252-1257 [PMID: 10220520 DOI: 10.1016/S0016-5085(99)70031-4]

118 **Vera-Portocarrero LP**, Lu Y, Westlund KN. Nociception in persistent pancreatitis in rats: effects of morphine and neuropeptide alterations. *Anesthesiology* 2003; **98**: 474-484 [PMID: 12552208 DOI: 10.1097/00000542-200302000-00029]

119 **Takamido S**, Kataoka Y, Tanano A, Cui Y, Ikeura T, Shimatani M, Kubota Y, Okazaki K, Yamada H. Intrapancreatic axonal hyperbranching of dorsal root ganglia neurons in chronic pancreatitis model rats and its relation to pancreatic pain. *Pancreas* 2006; **33**: 268-279 [PMID: 17003649 DOI: 10.1097/01.mpa.0000240600.72946.23]

120 **Wesselmann U**, Lai J. Mechanisms of referred visceral pain: uterine inflammation in the adult virgin rat results in neurogenic plasma extravasation in the skin. *Pain* 1997; **73**: 309-317 [PMID: 9469520 DOI: 10.1016/S0304-3959(97)00112-7]

121 **Winston JH**, He ZJ, Shenoy M, Xiao SY, Pasricha PJ. Molecular and behavioral changes in nociception in a novel rat model of chronic pancreatitis for the study of pain. *Pain* 2005; **117**: 214-222 [PMID: 16098667 DOI: 10.1016/j.pain.2005.06.013]

122 **Xu GY**, Winston JH, Shenoy M, Yin H, Pendyala S, Pasricha PJ. Transient receptor potential vanilloid 1 mediates hyperalgesia and is up-regulated in rats with chronic pancreatitis. *Gastroenterology* 2007; **133**: 1282-1292 [PMID: 17698068 DOI: 10.1053/j.gastro.2007.06.015]

123 **Zhang W**, Gao J, Zhao T, Wei L, Wu W, Bai Y, Zou D, Li Z.

Proteinase-activated receptor 2 mediates thermal hyperalgesia and is upregulated in a rat model of chronic pancreatitis. *Pancreas* 2011; **40**: 300-307 [PMID: 21311307 DOI: 10.1097/MPA.0b013e318201cbc1]

124 **Hughes MS**, Shenoy M, Liu L, Colak T, Mehta K, Pasricha PJ. Brain-derived neurotrophic factor is upregulated in rats with chronic pancreatitis and mediates pain behavior. *Pancreas* 2011; **40**: 551-556 [PMID: 21499209 DOI: 10.1097/MPA.0b013e318214fb77]

125 **Friess H**, Shrikhande S, Shrikhande M, Martignoni M, Kulli C, Zimmermann A, Kappeler A, Ramesh H, Büchler M. Neural alterations in surgical stage chronic pancreatitis are independent of the underlying aetiology. *Gut* 2002; **50**: 682-686 [PMID: 11950816 DOI: 10.1136/gut.50.5.682]

126 **Büchler M**, Weihe E, Friess H, Malfertheiner P, Bockman E, Müller S, Nohr D, Beger HG. Changes in peptidergic innervation in chronic pancreatitis. *Pancreas* 1992; **7**: 183-192 [PMID: 1372738 DOI: 10.1097/00006676-199203000-00009]

127 **Zhu ZW**, Friess H, Wang L, Zimmermann A, Büchler MW. Brain-derived neurotrophic factor (BDNF) is upregulated and associated with pain in chronic pancreatitis. *Dig Dis Sci* 2001; **46**: 1633-1639 [PMID: 11508661]

128 **Vera-Portocarrero LP**, Xie JY, Kowal J, Ossipov MH, King T, Porreca F. Descending facilitation from the rostral ventromedial medulla maintains visceral pain in rats with experimental pancreatitis. *Gastroenterology* 2006; **130**: 2155-2164 [PMID: 16762636 DOI: 10.1053/j.gastro.2006.03.025]

129 **Liu PY**, Lu CL, Wang CC, Lee IH, Hsieh JC, Chen CC, Lee HF, Lin HC, Chang FY, Lee SD. Spinal microglia initiate and maintain hyperalgesia in a rat model of chronic pancreatitis. *Gastroenterology* 2012; **142**: 165-173.e2 [PMID: 21963786 DOI: 10.1053/j.gastro.2011.09.041]

130 **Zhu Y**, Colak T, Shenoy M, Liu L, Pai R, Li C, Mehta K, Pasricha PJ. Nerve growth factor modulates TRPV1 expression and function and mediates pain in chronic pancreatitis. *Gastroenterology* 2011; **141**: 370-377 [PMID: 21473865 DOI: 10.1053/j.gastro.2011.03.046]

131 **Cattaruzza F**, Johnson C, Leggit A, Grady E, Schenk AK, Cevikbas F, Cedron W, Bondada S, Kirkwood R, Malone B, Steinhoff M, Bunnett N, Kirkwood KS. Transient receptor potential ankyrin 1 mediates chronic pancreatitis pain in mice. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G1002-G1012 [PMID: 23558009 DOI: 10.1152/ajpgi.00005.2013]

132 **Liao XZ**, Zhou MT, Mao YF, Xu H, Chen H, Sun JH, Xiong YC. Analgesic effects of gabapentin on mechanical hypersensitivity in a rat model of chronic pancreatitis. *Brain Res* 2010; **1337**: 104-112 [PMID: 20417627 DOI: 10.1016/j.brainres.2010.04.035]

P- Reviewer: Chow WK **S- Editor**: Gou SX **L- Editor**: A
E- Editor: Zhang DN

WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7231

World J Gastroenterol 2013 November 14; 19(42): 7231-7240
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Pathophysiology of chronic pancreatitis

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Supported by The Danish Council for Strategic Research

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Received: June 6, 2013 **Revised:** August 19, 2013
Accepted: August 28, 2013
Published online: November 14, 2013

Abstract

Chronic pancreatitis (CP) is an inflammatory disease of the pancreas characterized by progressive fibrotic destruction of the pancreatic secretory parenchyma. Despite the heterogeneity in pathogenesis and involved risk factors, processes such as necrosis/apoptosis, inflammation or duct obstruction are involved. This fibrosing process ultimately leads to progressive loss of the lobular morphology and structure of the pancreas, deformation of the large ducts and severe changes in the arrangement and composition of the islets. These conditions lead to irreversible morphological and structural changes resulting in impairment of both exocrine and endocrine functions. The prevalence of the disease is largely dependent on culture and geography. The etiological risk-factors associated with CP are multiple and involve both genetic and environmental factors. Throughout this review the M-ANNHEIM classification system will be used, comprising a detailed description of risk factors such as: alcohol-consumption, nicotine-consumption, nutritional factors, hereditary factors, efferent duct factors, immunological factors and miscellaneous and rare metabolic factors. Increased knowledge of the different etiological factors may encourage the use of further advanced diagnostic tools, which potentially will help clinicians to diagnose CP at an earlier stage. However, in view of the multi factorial disease and the complex clinical picture, it is not surprising that treatment of patients with CP is challenging and often unsuccessful.

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Key words: Chronic pancreatitis; Pathogenesis; Risk factors; Etiology

Core tip: The reported prevalence of chronic pancreatitis (CP) is approximately 0.5%. Etiological risk-factors associated with CP are multiple and throughout the review the M-ANNHEIM classification is used comprising environmental factors (alcohol consumption, nicotine habits and nutrition), hereditary, well characterized mutations, ductal obstruction and autoimmune factors. CP is characterized by progressive fibrotic destruction of glandular tissue, inflammation or duct obstruction, leading to irreversible functional impairment of both exocrine and endocrine functions. In view of the multifactorial disease and the complex clinical picture, it is not surprising that treatment of patients with CP is challenging and often unsuccessful.

Brock C, Nielsen LM, Lelic D, Drewes AM. Pathophysiology of chronic pancreatitis. *World J Gastroenterol* 2013; 19(42): 7231-7240 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7231.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7231

INTRODUCTION

The reported prevalence of chronic pancreatitis (CP) varies due to differences in study design, diagnostic criteria, culture and geography; however in Europe and United States it is relatively rare varying between 0.2% and 0.6%[1]. The annual incidence is estimated to be approximately 7-10 per 100000[2]. The etiological risk-factors associated with CP are multiple and involve environmental factors (alcohol consumption, nicotine habits and nutrition), hereditary well characterized mutations, ductal obstruction and autoimmune factors[3]. CP is characterized by progressive fibrotic destruction of the glandular tissue. The secretory parenchyma is destroyed by processes such as necrosis/apoptosis, inflammation or duct obstruction. Increasing evidence indicates that pancreatic stellate cells (PSC) are the major mediators of fibrosis, resulting in the formation of extracellular matrix (ECM) in the interstitial spaces and in the areas where acinar cells disappear or duct cells are injured. This process ultimately leads to progressive loss of the lobular morphology and structure of the pancreas, bizarre deformation of the large ducts and severe changes in the arrangement and composition of the islets. The fibrotic destruction of the pancreatic gland is irreversible and the morphological and structural changes lead to functional impairment of both exocrine and endocrine functions, eventually leading to malnutrition and/or diabetes[3-6]. In many aspects the PSCs share many similar features to hepatic stellate cells and glomerular mesangial cells. The onset of pancreatic fibrogenesis is caused by injury which may involve interstitial mesenchymal cells, the duct cells and/or the acinar cells. Which of these elements is affected depends on the etiological risk factor. However, destruction to any one of these pancreatic tissue compartments is associated with transformation of resident fibroblasts/pancreatic stellate cells into myofibroblast-like phenotypes; a process called activation. In the activated state PSCs express α-smooth muscle actin (α-SMA), proliferate, and secrete fibrillar collagens, including collagen Ⅰ and Ⅲ and fibronectin. All together this production and deposition of ECM is characteristic in chronic pancreatic fibrosis, and the PSCs likely represent the wound-healing myofibroblasts of the pancreas[7,8]. The exact pathophysiological mechanisms initiating and maintaining the development of fibrosis in the pancreas are poorly understood, but may be viewed as a progression similar to, *e.g.*, liver fibrosis[9]. Hence, the initial injury to one or all of the various tissue compartments or cell types of the pancreas, leads to cell necrosis and/or apoptosis and consequently release of cytokines/growth factors (*e.g.*, tumor growth factor b1, interleukin-8, platelet-derived growth factor and CC-chemokines), either from immigrating inflammatory cells, especially macrophages, and/or nearby preexistent epithelial or mesenchymal cells[10-13]. Thereafter damaged cells are phagocytosed by macrophages, causing release of cytokines, which in turn causes activation and proliferation of resident fibroblasts/PCS situated in the immediate surroundings of the original site of injury, which accordingly induces transformation into myofibroblast cells[7,14]. However, it has also been suggested as an alternative hypothesis, that the abovementioned progression is bypassed, and the initiating etiological factor (for instance, ethanol consumption) activates resident fibroblasts directly. In the final stage, myofibroblasts produce and extracellular matrix deposits replace the inflammatory infiltrate and affect the architecture and function of the remaining pancreatic tissues[4]. Subsequently, a vicious circle has started, because in order to facilitate the deposition of the newly formed ECM, myofibroblasts enhance production of specialized enzymes, such as metalloproteinase matrix metalloproteinase (MMP)-3 and MMP-9, which are able to demolish the normal pericellular ECM. These metalloproteinases are in return regulated by cytokine tumor growth factor (TGF)-β1s, which through autocrine inhibition enhances pancreatic fibrogenesis by reducing collagen degradation[8]. As a consequence of declined ECM production (due to withdrawal of the initiating factor), myofibroblasts may disappear either through apoptosis or retransformation into fibroblasts. Furthermore, pancreatic stellate cells express both mediators of matrix remodeling and the regulatory cytokine TGF-β1 that, by autocrine inhibition of MMP-3 and MMP-9, may enhance fibrogenesis by reducing collagen degradation[8]. Pancreatic stellate cells express both mediators of matrix remodeling and the regulatory cytokine TGF-β1 that, by autocrine inhibition of MMP-3 and MMP-9, may enhance fibrogenesis by reducing collagen degradation[8].

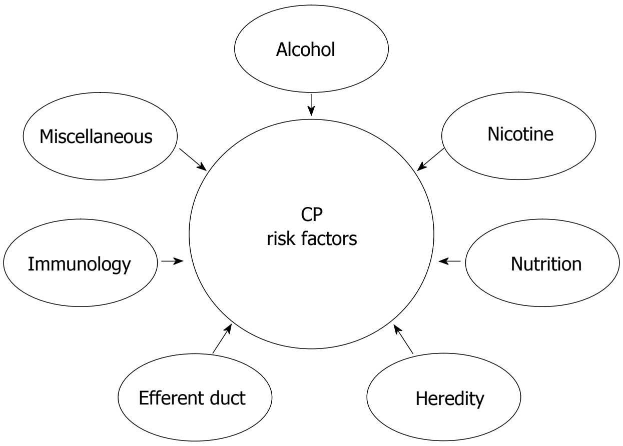

Figure 1 The etiological risk-factors associated with chronic pancreatitis are multiple and involve both genetic and environmental factors. According to the M-ANNHEIM classification system, different synergistic risk factors are known such as: alcohol-consumption, nicotine-consumption, nutritional factors, hereditary factors, efferent duct factors, immunological factors and miscellaneous and rare metabolic factors. CP: Chronic pancreatitis.

To describe the heterogeneity of the underlying pathophysiology we have throughout the review used the M-ANNHEIM classification[3], comprising detailed description of Multiple risk factors such as: alcohol-consumption, nicotine-consumption, nutritional factors, hereditary factors, efferent duct factors, immunological factors and miscellaneous including rare metabolic factors (Figure 1). Increased knowledge of the different etiological factors may encourage the use of further advanced diagnostic tools, which potentially will help clinicians to diagnose CP at an earlier stage.

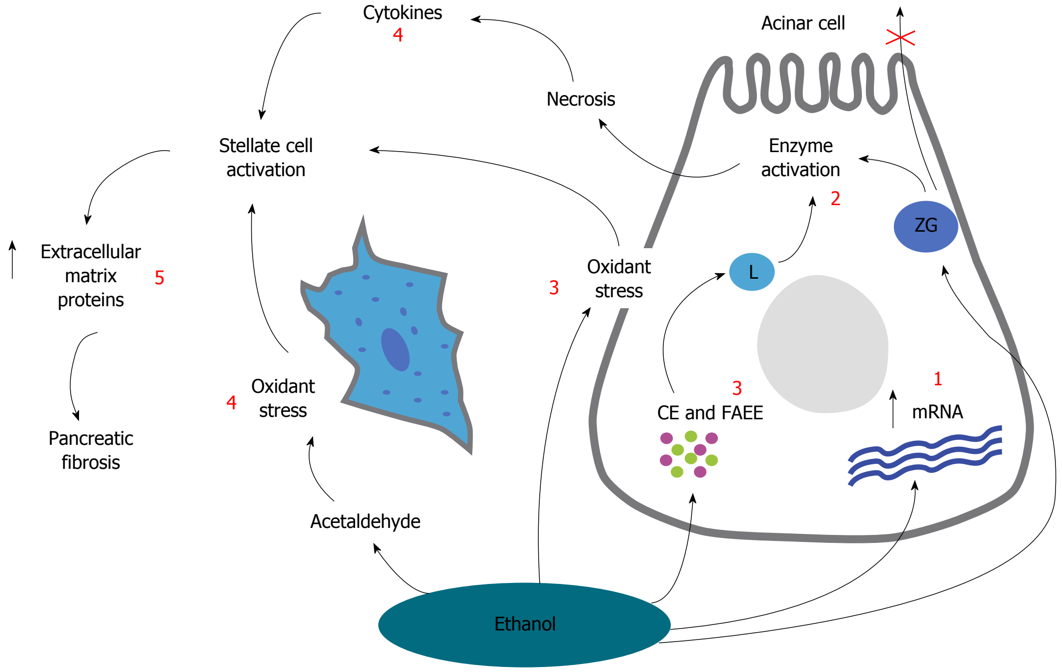

Figure 2 A schematic overview showing the overall hypothesis for the pathogenesis of alcoholic chronic pancreatitis. The effect of ethanol and its metabolites on the subcellular organelles include increased digestive and lysosomal enzyme content [due to increased synthesis (increased mRNA) and impaired secretion (1)] and destabilization of lysosomes (L) (2) and zymogen granules (ZG) [mediated by oxidant stress, cholesteryl esters (CE) and fatty acid ethyl esters (FAEE) (3)]. These changes will make the cell more sensitive to trigger factors and in the presence of appropriate trigger factors, overt acinar cell injury is initiated (alcoholic acute pancreatitis). Pancreatic stellate cells are activated by cytokines during alcohol-induced necroinflammation, or directly by ethanol *via* its metabolism to acetaldehyde and the subsequent generation of oxidant stress (4). Activated pancreatic stellate cell then increases the synthesis of extracellular matrix proteins leading to pancreatic fibrosis. Modified from Vonlaufen *et al*[84] (5).

However, in view of the multi-factorial disease and the complex clinical picture, it is not surprising that treatment of patients with CP is challenging and often unsuccessful.

According to the M-ANNHEIM classification, the following etiological risk factors are involved in the pathogenesis of chronic pancreatitis.

ALCOHOL CONSUMPTION

A relationship between alcohol consumption and pancreatic impairment has been reported as early as 1878[15] and hence, alcohol intake has long been regarded as the primary cause of chronic pancreatitis. Nowadays, there is satisfactory indication that the pancreas has the ability to metabolize ethanol *via* both oxidative and the non-oxidative pathways[16]. The metabolites and their byproducts injure acinal cells and activate stellate cells to produce and deposit ECM (Figure 2). A study by Dufour *et al*[17] suggested that CP development is associated to the dose and duration of alcohol consumption. It was estimated that approximately 80 g of alcohol per day for a minimum of 6-12 years is required to produce symptomatic pancreatitis. However, the consumption of lesser quantities may also lead to pancreatic injury and may have an impact on the progression of the disease[18]. In order to consider the risks associated with lower consumption of alcohol, the M-ANNHEIM classification system of CP grouped alcohol consumption into patterns of moderate (< 20 g pure ethanol per day), increased (20-80 g pure ethanol per day), or excessive (> 80 g pure ethanol per day)[3]. While alcohol consumption is doubtlessly a contributing factor in CP, it must be noted, that considerable amount of recent epidemiological studies and animal experiments suggest that alcohol alone is not sufficient to induce CP. An overview of the associations between alcohol consumption and other risk factors are listed in Table 1.

A multi-centre study from Italy reported that excessive alcohol consumption was the principal factor in only 34% of cases of CP[19] and an assessment from the United States concluded it was the main factor in 44% of CP cases[20]. Moreover, African-Americans are at particular risk of developing alcoholic CP[21]. Overall, less than 10% of heavy alcohol consumers develop alcoholic induced CP[17]. Hence, several theories have been proposed as to how alcohol might lead to CP, but no clear-cut answer exists as prolonged feeding of ethanol does not trigger the onset of CP in itself. Therefore, there are indications that alcohol sensitizes the pancreas to other external factors which interact to increase ethanol toxicity in vivo, such as cigarette smoking and diet[22] or genetic predisposition). Interestingly, alteration of pancreatic secretory trypsin inhibitor (*SPINK1*, Figure 3) and *CFTR* genes was present in patients with alcoholic CP and the increase of those genes was moreover associated with higher levels of alcohol consumption[23]. In this line, a recent study[24] found a genetic variant on chromosome X near the *CLDN2* gene

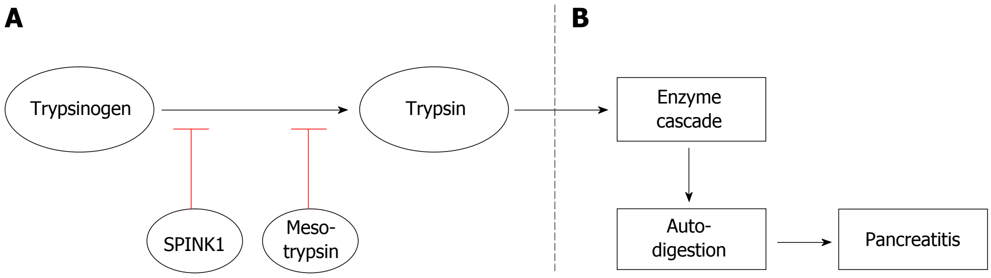

Figure 3 A scheme representing the role of digestive enzymes in normal pancreatic tissue and in the case of pancreatitis. A: In a normal pancreas, SPINK1 (first line of defense) and mesotrypsin (second line of defense) inhibit the generation of trypsin resulting from auto-activation of trypsinogen. These defense mechanisms prevent the pancreas from activating the pancreatic enzyme cascade and auto-digestion; B: If mutations are present in the SPINK1 and/or in the mesotrypsin gene, they losses their ability to inhibit the generation of trypsin resulting in activation of enzyme cascade and subsequent pancreatic auto-digestion leading to pancreatitis. SPINK1: Serine protease inhibitor, kazal type 1, gene (encodes for pancreatic secretory trypsin inhibitor).

Table 1 Association with alcoholic chronic pancreatitis

Factor	Association
Drink type	Yes: Vonlaufen *et al*[83] Nakamura *et al*[84] No: Levy *et al*[29] Wilson *et al*[85]
Drinking pattern	Yes: Lankisch *et al*[18] No: Levy *et al*[29] Wilson *et al*[85]
Diet	Yes: Levy *et al*[29] No: Wilson *et al*[85]
Tobacco	Yes: Rebours *et al*[28] Maisonneuve[27] Imoto[26] Lowenfels[86] No: Levy[29] Haber *et al*[87]
Genetics	Yes: Whitcomb *et al*[24] (PRSS1-PRSS2 and X- linked CLDN 2) Rosendahl[88] (Chymotrypsin C gene mutation) Miyasaka *et al*[89] (Cholesteryl esterlipase polymorphism) Ockenga *et al*[90] (UDP-glucuronosyl transferase) Witt *et al*[91] (SPINK1 mutations) No: Schneider *et al*[92] Perri *et al*[93] Frenzer *et al*[94] Schneider *et al*[82] Norton *et al*[95] Haber *et al*[96] Wilson *et al*[85]

PRSS1: Cationic trypsinogen gene; PRSS2: Anionic trypsinogen gene; CLDN 2: Claudin 2 gene; UDP-glucuronosyltransferase: Uridine 5´-diphospho-glucuronosyltransferase; SPINK1: Serine protease inhibitor, kazal type 1, gene (encodes for pancreatic secretory trypsin inhibitor).

that predicts which heavy drinking males were in higher risk of developing CP.

NICOTINE CONSUMPTION

Smoking has been identified as an important risk factor for development of chronic pancreatitis. The effect of tobacco in CP was first described in 1994[25], where heavy smokers had a significantly increased risk of developing pancreatic calcifications but no effects of alcohol were found. Another study reported that cigarette smoking increased the risk of pancreatic calcifications in late onset idiopathic CP in a population that never drank alcohol[26]. Moreover, a study by Maisonneuve *et al*[27] showed that smoking accelerated the degenerative alcohol induced pancreatitis quantified by the presence of calcifications and diabetes. Recently, a study reported that tobacco increased the occurrence of all major complications of alcoholic CP depending on the amount of smoking (1 pack-year is calculated as the number of cigarettes per day, multiplied by the number of years of smoking divided by 20 cigarettes/pack)[28]. No differences in CP outcome were seen at nicotine consumption corresponding to 10 pack-years. At a threshold of 15 pack-years CP was diagnosed earlier (36 years *vs* 46 years) and at nicotine consumption threshold of 20 pack-years up to 76% of patients were presented with pancreatic calcifications and ductal changes[28]. Tobacco effect on CP has long been considered to merely potentiate the main pancreatic toxic role of alcohol. However, although heavy smokers tended to be heavy drinkers, these recent findings indicate that tobacco intake is an independent risk factor in CP and accelerates the course of the disease.

NUTRITIONAL FACTORS

Only a few studies were conducted to investigate nutritional effect on CP[29-31], finding that diets rich in fat and protein possibly have an impact on the development of CP. However, due to subjective descriptions of daily nutritional customs and determination of the retrospective body mass index make it nearly impossible to deliver a simple report of past daily nutrition in majority of patients with CP. There is a type of CP named tropical CP which has been first described in Indonesia in 1959[32]. Tropical CP is a form of non-alcoholic CP existing in tropical developing countries. There is some confusion regarding the nomenclature sine tropical CP previously was hypothesized to be linked to diets high in consumption of cassava[33], which is a root high in carbohydrates but low in proteins and is cultivated in tropical and subtropical regions of the world. However, a study in rats which were fed cassava for up to one year failed to produce CP[34]. Moreover, a study in humans comparing the amount of consumption of cassava did not observe a difference between healthy controls and tropical CP patients[35], and today tropical pancreatitis is - at least partly - linked to genetic mutations[36]. Hence, tropical CP is widespread in parts of India and Africa where cassava is not a part of nutrition and tropical CP is not observed in rural West Africa where cassava intake is high[37]. Therefore, the cassava theory lacks evidence.

HEREDITARY FACTORS

Over the last decades an association between different types of chronic pancreatitis and hereditary factors has been reported. Hereditary chronic pancreatitis is a rare form which is characterized by an early onset of recurrent attacks of severe epigastric pain usually before ten years of age[38]. Back in 1952, Comfort and Steinberg[39] first described hereditary chronic pancreatitis as an autosomal dominant disease. In 1996, the hereditary chronic pancreatitis disease gene was mapped to chromosome 7q35, which encodes the cationic trypsinogen gene (*PRSS1*), and the first mutation, *R122H*, was detected[40]. Ever since then, many others mutations have been identified (*A16V, D22G, K23R, N29I, N29T, R122C* and *R122H)*[41-46] and nowadays it is known that approximately 80% of hereditary chronic pancreatitis patients have a *PRSS1* mutation[47]. *R122H* is the most frequent mutation causing hereditary chronic pancreatitis, where Arginine (Arg) is substituted with Histidine (His) at residue 117 on codon 122[5].

Trypsinogen is the inactive pro-enzyme for trypsin and cationic trypsinogen is the most abundant isoform in the human pancreatic juice (Figure 3). Trypsin becomes active when an eight-amino acid amino-terminal peptide is removed by an enteropeptidase, and the active trypsin plays a central role in pancreatic exocrine physiology as it initiates the cascade activation of other pancreatic digestive enzymes. Normally minor amounts of trypsin are activated within pancreatic acinar cells. Despite the small amount of active trypsin, an ongoing rapid inactivation takes place in order to prevent the digestion enzymes activation cascade and pancreatic auto digestion. To avoid auto digestion, two inhibitory mechanisms are present. First line of defense is the pancreatic secretory trypsin inhibitor (*PSTI*, gene name: serine protease inhibitor, kazal type 1, *SPINK1*), which inhibits up to 20 % of the trypsin activity[38,48]. If *SPINK1* fails to inhibit the trypsin activity, the trypsin-like enzymes (*e.g.,* mesotrypsin) are activated which hydrolyses trypsin and other zymogens (second line of defense)[49,50]. Hence, any mutation in *SPINK1* or the mesotrypsin gene will delay the protection, and therefore hereditary chronic pancreatitis patients are only protected against pancreatic auto digestion and progressive pancreatitis as long as the level of trypsin activity is less than *SPINK1* level.

In addition, a diagnosis of familial pancreatitis refers to pancreatitis from any cause that occurs in family with an incidence greater than expected by chance alone, given the size of the family and incidence of pancreatitis within a defined population[51]. Familial pancreatitis may as well be caused by genetic mutations.

As mentioned above *SPINK1* encodes *PSTI* (one of the defensive mechanisms against prematurely trypsin activity within the acinar cells) and *SPINK1* mutations are thought to be a disease modifying factor rather than being disease causing factor in idiopathic chronic pancreatitis[52,53].

Idiopathic chronic pancreatitis is defined as cases of pancreatitis within a family where no associated factor can be identified and represents 20%-30% of cases of chronic pancreatitis[54,55]. Most of the idiopathic chronic pancreatitis cases may be due to a variety of processes such as mutations in *SPINK1* and cystic fibrosis transmembrane conductance regulator (*CFTR*) gene[54-56], or Sjögren’s syndrome[57,58].

In 1998 a relationship between the *CFTR* gene and idiopathic chronic pancreatitis was described[59,60], but the underlying mechanisms leading to the development of chronic pancreatitis are still poorly understood. *CFTR* is identified as the cystic fibrosis gene and the link between cystic fibrosis and chronic pancreatitis is that both conditions may show abnormal sweat chloride concentrations together with pancreatic ductal obstruction caused by inspissated secretions. Furthermore, 1%-2% of patients with cystic fibrosis may suffer from recurrent pancreatitis[60,61], however up to 85% suffer from exocrine insufficiency and even up to 93% take exogenous pancreatic supplements[62].

There are two forms of idiopathic chronic pancreatitis: early- and late-onset pancreatitis, which both differ from alcoholic pancreatitis. Patients with early-onset develop calcification and exocrine and endocrine insufficiency more slowly than patients with late-onset disease, but they experience more severe pain[63].

EFFERENT DUCT FACTORS

Anatomically, the main pancreatic duct joins the common bile duct, after which both ducts perforate the medial side of the second portion of the duodenum at the major duodenal papilla. Hence any obstruction (partial or complete), compression or inflammation of the pancreatic tissue will increase the pressure within the pancreatic efferent ducts leading to ductal dilation proximal (upstream) of the stenosis and to atrophy of the acinar cells and replacement by fibrous tissue[4]. During embryogenesis, two efferent pancreatic ducts, a ventral and a dorsal duct fuse together to form one main pancreatic duct. When this fusion fails to occur, a pancreas divisum is formed, which is one of the most frequent congenital ductal anomalies. Theoretically this may cause flow problems within the pancreatic duct. However, pancreas divisum is present in up to 9% of autopsy studies and controversies exist whether presence of this abnormality is overrepresented in chronic pancreatitis in comparison to the normal population. Despite the rarity (5-15 cases per 100000), annular pancreas is another congenital disease which may be linked to linked to chronic pancreatitis, as the pancreatic tissue forms a complete or partial ring around the duodenum[64].

There are various other possible causes for a duct obstruction, but the most important and common is the narrowing and eventual occlusion of the main pancreatic duct in the head of the pancreas due to a ductal adenocarcinoma. Other causes for obstruction of the main pancreatic duct include intraductal papillary-mucinous neoplasms, cystic and endocrine neoplasms and acquired fibrous strictures. In the smaller pancreatic ducts, ductal papillary hyperplasia is a common cause of narrowing of the duct lumen. Finally, obstruction caused by a gallstones in the common bile duct (choledocholithiasis) or sphincter of Oddi dysmotility can obstruct flow and therefore cause retention of bile in the biliary tree and pancreatic juice in the pancreatic duct. The former is a frequent and well-known cause for acute pancreatitis.

IMMUNOLOGICAL RISK FACTORS

Immunological risk factors involved in chronic pancreatitis leading to autoimmune pancreatitis (AIP) deserve increased awareness as the underlying pathogenesis is not fully elucidated. The effort to diagnose autoimmune pancreatitis is mainly focused on the treatment possibilities with *e.g.*, glucocorticosteroids and differentiating the disease from pancreatic cancer. There are several lines of evidence that immunological factors play a key role in CP. Even though lymphoplasmacytic infiltration in AIP is most pronounced in the pancreatic ducts, advanced cases also show intralobular lymphoplasmacytic infiltration, and the inflammation may also specifically attack the acinar cells. IgG antibodies to a plasminogen-binding protein homologous to the human ubiquitin-protein ligase E3 component n-recognin 2, is expressed in pancreatic acinar cells, and have been found in the sera of AIP patients[65]. In AIP patients auto-antibodies to trypsinogens are also upregulated, which is not the case in alcohol induced chronic pancreatitis patients, corresponding to loss of trypsinogen-positive acinar cells in AIP tissues[66]. Furthermore, basement membrane deposits of complement C3c, IgG4 and IgG not only around pancreatic ducts but also around acini have been demonstrated in AIP[67].

Even though it has not yet been fully proved that there are differences in the pathogenesis, AIP is separated into two distinct types: Type 1 and type 2 AIP.

Type 1 AIP patients (most common type in East Asia) are typically older than type 2 patients, with a mean age at disease onset of 62 years[68]. Plasma levels of IgG4 are increased and in approximately 50% of the cases other organs are affected[68,69]. The key histological feature that distinguishes type 1 AIP from type 2 AIP is strong infiltration of IgG4-immunopositive plasma cells and lymphoplasmacytic infiltration[70,71] IgG4-positive sclerosing cholangitis represents one of the main extrapancreatic manifestations of type 1 AIP, and hence jaundice is a common symptom[72]. Due to the multiple extrapancreatic manifestations of type 1 AIP, Kamisawa *et al*[73] promoted the concept of AIP as part of a clinicopathological entity of IgG4-associated systemic disease, together with retroperitoneal fibrosis, sclerosing sialadenitis and sclerosing cholangitis. This systemic fibroinflammatory disease probably also includes lesions in the aorta, breast and prostate[73-76].

Type 2 AIP patients (45% of the cases in United States and Europe) are typically younger than type 1 AIP, with a mean age of 40-48 years[68,77]. AIP is often associated with Crohn's disease and ulcerative colitis[78]. The most characteristic histological feature which distinguishes type 2 from type 1 AIP is the so-called granulocytic epithelial lesions which are a hallmark of type 2 AIP[77,79]. Besides, type 2 AIP is usually not associated with the extrapancreatic manifestations observed in type 1 AIP.

MISCELLANEOUS

Tropical chronic pancreatitis

Tropical chronic pancreatitis may be referred to an idiopathic, juvenile, non-alcoholic form of chronic pancreatitis widely prevalent in the developing countries of the tropical world. Tropical chronic pancreatitis can be sub-grouped in two entities: Tropical calcific pancreatitis and fibrocalculous pancreatic diabetes. Tropical calcific pancreatitis describes the early pre-diabetic stage of the disease and affects younger people. Tropical calcific pancreatitis is characterized by severe abdominal pain, pancreatic calcification, and signs of pancreatic dysfunction (no diabetes mellitus at the time of diagnosis). Fibrocalculous pancreatic diabetes describes the late diabetic stage of the disease where diabetes mellitus is the first major clinical sign to determine the diagnosis of fibrocalculous pancreatic diabetes. The etiology of tropical calcific pancreatitis and fibrocalculous pancreatic diabetes has shown to be related to genetic mutations in the *SPINK1* gene, but it is still unknown if environmental factors have an influence[36,80-82].

Primary hypercalcemia

It has been suggested that higher serum calcium levels may contribute to pancreatitis, as hypercalcemia may predispose the pancreatic acinar cell to abnormal, sustained calcium levels, which leads to premature pancreatic protease activation, and consequently pancreatitis.

Hyperparathyreoidisme

Controversies exist regarding associations between primary hyperparathyroidism and acute or chronic pancreatitis. However, most studies show an increased rate of pancreatitis among patients with primary hyperparathyroidism in comparison to general hospitalized patients without the disease.

Hyperlipidemia

Hyperlipidemia covers abnormally elevated levels of any or all lipids, lipoproteins or both in the blood. Hyperlipidemia can be divided in primary and secondary subtypes. Lipid and lipoprotein abnormalities are relatively common in the general population, and are regarded as a modifiable risk factor associated with acute pancreatitis.

CONCLUSION

Increased knowledge of different etiological factors and how they interact may encourage the use of further advanced diagnostic tools, which potentially will help clinicians to diagnose and treat CP at an earlier stage. Recent research has increased our understanding of the disease and has changed the approach to CP. However, in view of the multi-factorial disease and the complex clinical picture, it is not surprising that treatment of patients with CP is challenging and often unsuccessful.

REFERENCES

1 **Lévy P**, Barthet M, Mollard BR, Amouretti M, Marion-Audibert AM, Dyard F. Estimation of the prevalence and incidence of chronic pancreatitis and its complications. *Gastroenterol Clin Biol* 2006; **30**: 838-844 [PMID: 16885867 DOI: 10.1016/S0399-8320(06)73330-9]

2 **Andersen BN**, Pedersen NT, Scheel J, Worning H. Incidence of alcoholic chronic pancreatitis in Copenhagen. *Scand J Gastroenterol* 1982; **17**: 247-252 [PMID: 7134849 DOI: 10.3109/00365528209182047]

3 **Schneider A**, Löhr JM, Singer MV. The M-ANNHEIM classification of chronic pancreatitis: introduction of a unifying classification system based on a review of previous classifications of the disease. *J Gastroenterol* 2007; **42**: 101-119 [PMID: 17351799 DOI: 10.1007/s00535-006-1945-4]

4 **Klöppel G**, Detlefsen S, Feyerabend B. Fibrosis of the pancreas: the initial tissue damage and the resulting pattern. *Virchows Arch* 2004; **445**: 1-8 [PMID: 15138818]

5 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244 DOI: 10.1053/gast.2001.22586]

6 **Andrén-Sandberg A**, Hoem D, Gislason H. Pain management in chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2002; **14**: 957-970 [PMID: 12352215 DOI: 10.1097/00042737-200209000-00006]

7 **Apte MV**, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. *Gut* 1999; **44**: 534-541 [PMID: 10075961 DOI: 10.1136/gut.44.4.534]

8 **Shek FW**, Benyon RC, Walker FM, McCrudden PR, Pender SL, Williams EJ, Johnson PA, Johnson CD, Bateman AC, Fine DR, Iredale JP. Expression of transforming growth factor-beta 1 by pancreatic stellate cells and its implications for matrix secretion and turnover in chronic pancreatitis. *Am J Pathol* 2002; **160**: 1787-1798 [PMID: 12000730 DOI: 10.1016/S0002-9440(10)61125-X]

9 **Bedossa P**, Paradis V. Liver extracellular matrix in health and disease. *J Pathol* 2003; **200**: 504-515

10 **Andoh A**, Takaya H, Saotome T, Shimada M, Hata K, Araki Y, Nakamura F, Shintani Y, Fujiyama Y, Bamba T. Cytokine regulation of chemokine (IL-8, MCP-1, and RANTES) gene expression in human pancreatic periacinar myofibroblasts. *Gastroenterology* 2000; **119**: 211-219 [PMID: 10889171 DOI: 10.1053/gast.2000.8538]

11 **Ebert M**, Kasper HU, Hernberg S, Friess H, Büchler MW, Roessner A, Korc M, Malfertheiner P. Overexpression of platelet-derived growth factor (PDGF) B chain and type beta PDGF receptor in human chronic pancreatitis. *Dig Dis Sci* 1998; **43**: 567-574 [PMID: 9539653 DOI: 10.1023/A:1018867209170]

12 **Luttenberger T**, Schmid-Kotsas A, Menke A, Siech M, Beger H, Adler G, Grünert A, Bachem MG. Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: implications in pathogenesis of pancreas fibrosis. *Lab Invest* 2000; **80**: 47-55 [PMID: 10653002 DOI: 10.1038/labinvest.3780007]

13 **Saurer L**, Reber P, Schaffner T, Büchler MW, Buri C, Kappeler A, Walz A, Friess H, Mueller C. Differential expression of chemokines in normal pancreas and in chronic pancreatitis. *Gastroenterology* 2000; **118**: 356-367 [PMID: 10648464 DOI: 10.1016/S0016-5085(00)70218-6]

14 **Haber PS**, Keogh GW, Apte MV, Moran CS, Stewart NL, Crawford DH, Pirola RC, McCaughan GW, Ramm GA, Wilson JS. Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis. *Am J Pathol* 1999; **155**: 1087-1095 [PMID: 10514391 DOI: 10.1016/S0002-9440(10)65211-X]

15 **Friedreich N**. Disease of the pancreas. Cyclopoedia of the Practice of Medicine. New York: William Wood, 1878

16 **Vonlaufen A**, Wilson JS, Pirola RC, Apte MV. Role of alcohol metabolism in chronic pancreatitis. *Alcohol Res Health* 2007; **30**: 48-54 [PMID: 17718401]

17 **Dufour MC**, Adamson MD. The epidemiology of alcohol-induced pancreatitis. *Pancreas* 2003; **27**: 286-290 [PMID: 14576488 DOI: 10.1097/00006676-200311000-00002]

18 **Lankisch MR**, Imoto M, Layer P, DiMagno EP. The effect of small amounts of alcohol on the clinical course of chronic pancreatitis. *Mayo Clin Proc* 2001; **76**: 242-251 [PMID: 11243270 DOI: 10.4065/76.3.242]

19 **Frulloni L**, Gabbrielli A, Pezzilli R, Zerbi A, Cavestro GM, Marotta F, Falconi M, Gaia E, Uomo G, Maringhini A, Mutignani M, Maisonneuve P, Di Carlo V, Cavallini G. Chronic pancreatitis: report from a multicenter Italian survey (PanCroInfAISP) on 893 patients. *Dig Liver Dis* 2009; **41**: 311-317 [PMID: 19097829 DOI: 10.1016/j.dld.2008.07.316]

20 **Yadav D**, Whitcomb DC. The role of alcohol and smoking in pancreatitis. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 131-145 [PMID: 20125091 DOI: 10.1038/nrgastro.2010.6]

21 **Herreros-Villanueva M**, Hijona E, Bañales JM, Cosme A, Bujanda L. Alcohol consumption on pancreatic diseases. *World J Gastroenterol* 2013; **19**: 638-647 [PMID: 23429423 DOI: 10.3748/wjg.v19.i5.638]

22 **Pandol SJ**, Gukovsky I, Satoh A, Lugea A, Gukovskaya AS. Emerging concepts for the mechanism of alcoholic pancreatitis from experimental models. *J Gastroenterol* 2003; **38**: 623-628 [PMID: 12898353 DOI: 10.1007/s00535-003-1134-7]

23 **Keim V**. [Chronic pancreatitis--pancreas cancer: influence of genetic factors]. *Praxis (Bern 1994)* 2005; **94**: 811-817 [PMID: 15957615]

24 **Whitcomb DC**, LaRusch J, Krasinskas AM, Klei L, Smith JP, Brand RE, Neoptolemos JP, Lerch MM, Tector M, Sandhu BS, Guda NM, Orlichenko L, Alkaade S, Amann ST, Anderson MA, Baillie J, Banks PA, Conwell D, Coté GA, Cotton PB, DiSario J, Farrer LA, Forsmark CE, Johnstone M, Gardner TB, Gelrud A, Greenhalf W, Haines JL, Hartman DJ, Hawes RA, Lawrence C, Lewis M, Mayerle J, Mayeux R, Melhem NM, Money ME, Muniraj T, Papachristou GI, Pericak-Vance MA, Romagnuolo J, Schellenberg GD, Sherman S, Simon P, Singh VP, Slivka A, Stolz D, Sutton R, Weiss FU, Wilcox CM, Zarnescu NO, Wisniewski SR, O'Connell MR, Kienholz ML, Roeder K, Barmada MM, Yadav D, Devlin B. Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. *Nat Genet* 2012; **44**: 1349-1354 [PMID: 23143602 DOI: 10.1038/ng.2466]

25 **Cavallini G**, Talamini G, Vaona B, Bovo P, Filippini M, Rigo

L, Angelini G, Vantini I, Riela A, Frulloni L. Effect of alcohol and smoking on pancreatic lithogenesis in the course of chronic pancreatitis. *Pancreas* 1994; **9**: 42-46 [PMID: 8108370 DOI: 10.1097/00006676-199401000-00006]

26 **Imoto M**, DiMagno EP. Cigarette smoking increases the risk of pancreatic calcification in late-onset but not early-onset idiopathic chronic pancreatitis. *Pancreas* 2000; **21**: 115-119 [PMID: 10975703 DOI: 10.1097/00006676-200008000-00002]

27 **Maisonneuve P**, Lowenfels AB, Müllhaupt B, Cavallini G, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L, Frulloni L, Ammann RW. Cigarette smoking accelerates progression of alcoholic chronic pancreatitis. *Gut* 2005; **54**: 510-514 [PMID: 15753536 DOI: 10.1136/gut.2004.039263]

28 **Rebours V**, Vullierme MP, Hentic O, Maire F, Hammel P, Ruszniewski P, Lévy P. Smoking and the course of recurrent acute and chronic alcoholic pancreatitis: a dose-dependent relationship. *Pancreas* 2012; **41**: 1219-1224 [PMID: 23086245 DOI: 10.1097/MPA.0b013e31825de97d]

29 **Lévy P**, Mathurin P, Roqueplo A, Rueff B, Bernades P. A multidimensional case-control study of dietary, alcohol, and tobacco habits in alcoholic men with chronic pancreatitis. *Pancreas* 1995; **10**: 231-238 [PMID: 7624300 DOI: 10.1097/00006676-199504000-00003]

30 **Grendell JH**. Nutrition and absorption in diseases of the pancreas. *Clin Gastroenterol* 1983; **12**: 551-562 [PMID: 6347468]

31 **Uscanga L**, Robles-Díaz G, Sarles H. Nutritional data and etiology of chronic pancreatitis in Mexico. *Dig Dis Sci* 1985; **30**: 110-113 [PMID: 3967558 DOI: 10.1007/BF01308194]

32 **Zuidema PJ**. Cirrhosis and disseminated calcification of the pancreas in patients with malnutrition. *Trop Geogr Med* 1959; **11**: 70-74 [PMID: 13659585]

33 **Barman KK**, Premalatha G, Mohan V. Tropical chronic pancreatitis. *Postgrad Med J* 2003; **79**: 606-615 [PMID: 14654569 DOI: 10.1136/pmj.79.937.606]

34 **Mathangi DC**, Deepa R, Mohan V, Govindarajan M, Namasivayam A. Long-term ingestion of cassava (tapioca) does not produce diabetes or pancreatitis in the rat model. *Int J Pancreatol* 2000; **27**: 203-208 [PMID: 10952402 DOI: 10.1385/IJGC]

35 **Balakrishnan V**, Hariharan M, Sindhu S. Pathogenesis of pancreatitis in India leading on to diabetes mellitus. *Proc Nutr Soc India* 1990; **36**: 1-7

36 **Chandak GR**, Idris MM, Reddy DN, Bhaskar S, Sriram PV, Singh L. Mutations in the pancreatic secretory trypsin inhibitor gene (PSTI/SPINK1) rather than the cationic trypsinogen gene (PRSS1) are significantly associated with tropical calcific pancreatitis. *J Med Genet* 2002; **39**: 347-351 [PMID: 12011155]

37 **Teuscher T**, Baillod P, Rosman JB, Teuscher A. Absence of diabetes in a rural West African population with a high carbohydrate/cassava diet. *Lancet* 1987; **1**: 765-768 [PMID: 2882181 DOI: 10.1016/S0140-6736(87)92797-8]

38 **Whitcomb DC**, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996; **14**: 141-145 [PMID: 8841182 DOI: 10.1038/ng1096-141]

39 **Comfort MW**, Steinberg AG. Pedigree of a family with hereditary chronic relapsing pancreatitis. *Gastroenterology* 1952; **21**: 54-63 [PMID: 14926813]

40 **Whitcomb DC**, Preston RA, Aston CE, Sossenheimer MJ, Barua PS, Zhang Y, Wong-Chong A, White GJ, Wood PG, Gates LK, Ulrich C, Martin SP, Post JC, Ehrlich GD. A gene for hereditary pancreatitis maps to chromosome 7q35. *Gastroenterology* 1996; **110**: 1975-1980 [PMID: 8964426 DOI: 10.1053/gast.1996.v110.pm8964426]

41 **Gorry MC**, Gabbaizedeh D, Furey W, Gates LK, Preston RA, Aston CE, Zhang Y, Ulrich C, Ehrlich GD, Whitcomb DC. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology* 1997; **113**: 1063-1068 [PMID: 9322498 DOI: 10.1053/gast.1997.v113.pm9322498]

42 **Férec C**, Raguénès O, Salomon R, Roche C, Bernard JP, Guillot M, Quéré I, Faure C, Mercier B, Audrézet MP, Guillausseau PJ, Dupont C, Munnich A, Bignon JD, Le Bodic L. Mutations in the cationic trypsinogen gene and evidence for genetic heterogeneity in hereditary pancreatitis. *J Med Genet* 1999; **36**: 228-232 [PMID: 10204851]

43 **Witt H**, Luck W, Becker M. A signal peptide cleavage site mutation in the cationic trypsinogen gene is strongly associated with chronic pancreatitis. *Gastroenterology* 1999; **117**: 7-10 [PMID: 10381903 DOI: 10.1016/S0016-5085(99)70543-3]

44 **Pfützer R**, Myers E, Applebaum-Shapiro S, Finch R, Ellis I, Neoptolemos J, Kant JA, Whitcomb DC. Novel cationic trypsinogen (PRSS1) N29T and R122C mutations cause autosomal dominant hereditary pancreatitis. *Gut* 2002; **50**: 271-272 [PMID: 11788572 DOI: 10.1136/gut.50.2.271]

45 **Simon P**, Weiss FU, Sahin-Toth M, Parry M, Nayler O, Lenfers B, Schnekenburger J, Mayerle J, Domschke W, Lerch MM. Hereditary pancreatitis caused by a novel PRSS1 mutation (Arg-122 --& gt; Cys) that alters autoactivation and autodegradation of cationic trypsinogen. *J Biol Chem* 2002; **277**: 5404-5410 [PMID: 11719509 DOI: 10.1074/jbc.M108073200]

46 **Rebours V**, Lévy P, Ruszniewski P. An overview of hereditary pancreatitis. *Dig Liver Dis* 2012; **44**: 8-15 [PMID: 21907651 DOI: 10.1016/j.dld.2011.08.003]

47 **Joergensen MT**, Brusgaard K, Crüger DG, Gerdes AM, Schaffalitzky de Muckadell OB. Genetic, epidemiological, and clinical aspects of hereditary pancreatitis: a population-based cohort study in Denmark. *Am J Gastroenterol* 2010; **105**: 1876-1883 [PMID: 20502448 DOI: 10.1038/ajg.2010.193]

48 **Yamamoto T**, Nakamura Y, Nishide J, Emi M, Ogawa M, Mori T, Matsubara K. Molecular cloning and nucleotide sequence of human pancreatic secretory trypsin inhibitor (PSTI) cDNA. *Biochem Biophys Res Commun* 1985; **132**: 605-612 [PMID: 3877508 DOI: 10.1016/0006-291X(85)91176-3]

49 **Rinderknecht H**, Renner IG, Abramson SB, Carmack C. Mesotrypsin: a new inhibitor-resistant protease from a zymogen in human pancreatic tissue and fluid. *Gastroenterology* 1984; **86**: 681-692 [PMID: 6698368]

50 **Rinderknecht H**, Adham NF, Renner IG, Carmack C. A possible zymogen self-destruct mechanism preventing pancreatic autodigestion. *Int J Pancreatol* 1988; **3**: 33-44 [PMID: 3162506]

51 **Whitcomb DC**. Hereditary diseases of the pancreas. In: Yamada T, Alpers DH, Kaplowitz N, Laine L, Owyang C, Powell DW, editors. Textbook of Gastroenterology. 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2003: 2147-2165

52 **Pfützer RH**, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J, Furey WF, Whitcomb DC. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 2000; **119**: 615-623 [PMID: 10982753 DOI: 10.1053/gast.2000.18017]

53 **Chen JM**, Férec C. Chronic pancreatitis: genetics and pathogenesis. *Annu Rev Genomics Hum Genet* 2009; **10**: 63-87 [PMID: 19453252 DOI: 10.1146/annurev-genom-082908-150009]

54 **Naruse S**, Kitagawa M, Ishiguro H. Molecular understanding of chronic pancreatitis: a perspective on the future. *Mol Med Today* 1999; **5**: 493-499 [PMID: 10529791 DOI: 10.1016/S1357-4310(99)01595-6]

55 **Cohn JA**. Reduced CFTR function and the pathobiology of idiopathic pancreatitis. *J Clin Gastroenterol* 2005; **39**: S70-S77 [PMID: 15758663 DOI: 10.1097/01.mcg.0000155522.89005.bf]

56 **Midha S**, Khajuria R, Shastri S, Kabra M, Garg PK. Idiopathic chronic pancreatitis in India: phenotypic characterisation and strong genetic susceptibility due to SPINK1 and CFTR gene mutations. *Gut* 2010; **59**: 800-807 [PMID: 20551465 DOI:

10.1136/gut.2009.191239]

57 **Onodera M**, Okazaki K, Morita M, Nishimori I, Yamamoto Y. Immune complex specific for the pancreatic duct antigen in patients with idiopathic chronic pancreatitis and Sjögren syndrome. *Autoimmunity* 1994; **19**: 23-29 [PMID: 7749039 DOI: 10.3109/08916939409008005]

58 **Nishimori I**, Yamamoto Y, Okazaki K, Morita M, Onodera M, Kino J, Tamura S, Yamamoto Y. Identification of autoantibodies to a pancreatic antigen in patients with idiopathic chronic pancreatitis and Sjögren's syndrome. *Pancreas* 1994; **9**: 374-381 [PMID: 8022761 DOI: 10.1097/00006676-199405000-00015]

59 **Sharer N**, Schwarz M, Malone G, Howarth A, Painter J, Super M, Braganza J. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med* 1998; **339**: 645-652 [PMID: 9725921 DOI: 10.1056/NEJM199809033391001]

60 **Atlas AB**, Orenstein SR, Orenstein DM. Pancreatitis in young children with cystic fibrosis. *J Pediatr* 1992; **120**: 756-759 [PMID: 1578312 DOI: 10.1016/S0022-3476(05)80242-2]

61 **Shwachman H**, Lebenthal E, Khaw KT. Recurrent acute pancreatitis in patients with cystic fibrosis with normal pancreatic enzymes. *Pediatrics* 1975; **55**: 86-95 [PMID: 1110867]

62 **Borowitz D**, Baker SS, Duffy L, Baker RD, Fitzpatrick L, Gyamfi J, Jarembek K. Use of fecal elastase-1 to classify pancreatic status in patients with cystic fibrosis. *J Pediatr* 2004; **145**: 322-326 [PMID: 15343184 DOI: 10.1016/j.jpeds.2004.04.049]

63 **Layer P**, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology* 1994; **107**: 1481-1487 [PMID: 7926511 DOI: 10.1016/0016-5085(94)90553-3]

64 **Tas A**, Köklü S, Kocak E, Akbal E, Ergul B. An unusual cause of acute pancreatitis: annular pancreas and papillary opening of the cystic duct. *Gut Liver* 2012; **6**: 403-404 [PMID: 22844573 DOI: 10.5009/gnl.2012.6.3.403]

65 **Frulloni L**, Lunardi C, Simone R, Dolcino M, Scattolini C, Falconi M, Benini L, Vantini I, Corrocher R, Puccetti A. Identification of a novel antibody associated with autoimmune pancreatitis. *N Engl J Med* 2009; **361**: 2135-2142 [PMID: 19940298 DOI: 10.1056/NEJMoa0903068]

66 **Löhr JM**, Faissner R, Koczan D, Bewerunge P, Bassi C, Brors B, Eils R, Frulloni L, Funk A, Halangk W, Jesenofsky R, Kaderali L, Kleeff J, Krüger B, Lerch MM, Lösel R, Magnani M, Neumaier M, Nittka S, Sahin-Tóth M, Sänger J, Serafini S, Schnölzer M, Thierse HJ, Wandschneider S, Zamboni G, Klöppel G. Autoantibodies against the exocrine pancreas in autoimmune pancreatitis: gene and protein expression profiling and immunoassays identify pancreatic enzymes as a major target of the inflammatory process. *Am J Gastroenterol* 2010; **105**: 2060-2071 [PMID: 20407433 DOI: 10.1038/ajg.2010.141]

67 **Detlefsen S**, Bräsen JH, Zamboni G, Capelli P, Klöppel G. Deposition of complement C3c, immunoglobulin (Ig)G4 and IgG at the basement membrane of pancreatic ducts and acini in autoimmune pancreatitis. *Histopathology* 2010; **57**: 825-835 [PMID: 21166697 DOI: 10.1111/j.1365-2559.2010.03717.x]

68 **Sah RP**, Chari ST, Pannala R, Sugumar A, Clain JE, Levy MJ, Pearson RK, Smyrk TC, Petersen BT, Topazian MD, Takahashi N, Farnell MB, Vege SS. Differences in clinical profile and relapse rate of type 1 versus type 2 autoimmune pancreatitis. *Gastroenterology* 2010; **139**: 140-18; quiz 140-18; [PMID: 20353791 DOI: 10.1053/j.gastro.2010.03.054]

69 **Chari ST**, Longnecker DS, Klöppel G. The diagnosis of autoimmune pancreatitis: a Western perspective. *Pancreas* 2009; **38**: 846-848 [PMID: 19855232 DOI: 10.1097/MPA.0b013e3181bba281]

70 **Kamisawa T**, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroenterol* 2006; **41**: 613-625 [PMID: 16932997 DOI: 10.1007/s00535-006-1862-6]

71 **Gardner TB**, Chari ST. Autoimmune pancreatitis. *Gastroenterol Clin North Am* 2008; **37**: 439-60, vii [PMID: 18499030 DOI: 10.1016/j.gtc.2008.02.004]

72 **Kamisawa T**, Anjiki H, Egawa N. Rapid changes in sclerosing cholangitis associated with autoimmune pancreatitis. *Pancreas* 2009; **38**: 601-602 [PMID: 19550281 DOI: 10.1097/MPA.0b013e31819e0cdb]

73 **Cheuk W**, Chan AC, Lam WL, Chow SM, Crowley P, Lloydd R, Campbell I, Thorburn M, Chan JK. IgG4-related sclerosing mastitis: description of a new member of the IgG4-related sclerosing diseases. *Am J Surg Pathol* 2009; **33**: 1058-1064 [PMID: 19384187 DOI: 10.1097/PAS.0b013e3181998cbe]

74 **Kasashima S**, Zen Y, Kawashima A, Endo M, Matsumoto Y, Kasashima F. A new clinicopathological entity of IgG4-related inflammatory abdominal aortic aneurysm. *J Vasc Surg* 2009; **49**: 1264-171; discussion 1271 [PMID: 19217746 DOI: 10.1016/j.jvs.2008.11.072]

75 **Sakata N**, Tashiro T, Uesugi N, Kawara T, Furuya K, Hirata Y, Iwasaki H, Kojima M. IgG4-positive plasma cells in inflammatory abdominal aortic aneurysm: the possibility of an aortic manifestation of IgG4-related sclerosing disease. *Am J Surg Pathol* 2008; **32**: 553-559 [PMID: 18300798 DOI: 10.1097/PAS.0b013e31815a04db]

76 **Uehara T**, Hamano H, Kawakami M, Koyama M, Kawa S, Sano K, Honda T, Oki K, Ota H. Autoimmune pancreatitis-associated prostatitis: distinct clinicopathological entity. *Pathol Int* 2008; **58**: 118-125 [PMID: 18199162 DOI: 10.1111/j.1440-1827.2007.02199.x]

77 **Zamboni G**, Lüttges J, Capelli P, Frulloni L, Cavallini G, Pederzoli P, Leins A, Longnecker D, Klöppel G. Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: a study on 53 resection specimens and 9 biopsy specimens. *Virchows Arch* 2004; **445**: 552-563 [PMID: 15517359 DOI: 10.1007/s00428-004-1140-z]

78 **Klöppel G**, Detlefsen S, Chari ST, Longnecker DS, Zamboni G. Autoimmune pancreatitis: the clinicopathological characteristics of the subtype with granulocytic epithelial lesions. *J Gastroenterol* 2010; **45**: 787-793 [PMID: 20549251 DOI: 10.1007/s00535-010-0265-x]

79 **Detlefsen S**, Sipos B, Zhao J, Drewes AM, Klöppel G. Autoimmune pancreatitis: expression and cellular source of profibrotic cytokines and their receptors. *Am J Surg Pathol* 2008; **32**: 986-995 [PMID: 18460977 DOI: 10.1097/PAS.0b013e31815d2583]

80 **Hassan Z**, Mohan V, Ali L, Allotey R, Barakat K, Faruque MO, Deepa R, McDermott MF, Jackson AE, Cassell P, Curtis D, Gelding SV, Vijayaravaghan S, Gyr N, Whitcomb DC, Khan AK, Hitman GA. SPINK1 is a susceptibility gene for fibrocalculous pancreatic diabetes in subjects from the Indian subcontinent. *Am J Hum Genet* 2002; **71**: 964-968 [PMID: 12187509 DOI: 10.1086/342731]

81 **Bhatia E**, Choudhuri G, Sikora SS, Landt O, Kage A, Becker M, Witt H. Tropical calcific pancreatitis: strong association with SPINK1 trypsin inhibitor mutations. *Gastroenterology* 2002; **123**: 1020-1025 [PMID: 12360463 DOI: 10.1053/gast.2002.36028]

82 **Schneider A**, Suman A, Rossi L, Barmada MM, Beglinger C, Parvin S, Sattar S, Ali L, Khan AK, Gyr N, Whitcomb DC. SPINK1/PSTI mutations are associated with tropical pancreatitis and type II diabetes mellitus in Bangladesh. *Gastroenterology* 2002; **123**: 1026-1030 [PMID: 12360464 DOI: 10.1053/gast.2002.36059]

83 **Vonlaufen A**, Wilson JS, Apte MV. Molecular mechanisms of pancreatitis: current opinion. *J Gastroenterol Hepatol* 2008; **23**: 1339-1348 [PMID: 18853993 DOI: 10.1111/j.1440-1746.2008.05520.x]

84 **Nakamura Y**, Kobayashi Y, Ishikawa A, Maruyama K, Higuchi S. Severe chronic pancreatitis and severe liver cirrhosis

have different frequencies and are independent risk factors in male Japanese alcoholics. *J Gastroenterol* 2004; **39**: 879-887 [PMID: 15565408 DOI: 10.1007/s00535-004-1405-y]

85 **Wilson JS**, Bernstein L, McDonald C, Tait A, McNeil D, Pirola RC. Diet and drinking habits in relation to the development of alcoholic pancreatitis. *Gut* 1985; **26**: 882-887 [PMID: 4029715 DOI: 10.1136/gut.26.9.882]

86 **Lowenfels AB**, Zwemer FL, Jhangiani S, Pitchumoni CS. Pancreatitis in a native American Indian population. *Pancreas* 1987; **2**: 694-697 [PMID: 3438307 DOI: 10.1097/00006676-198711000-00012]

87 **Haber PS**, Wilson JS, Pirola RC. Smoking and alcoholic pancreatitis. *Pancreas* 1993; **8**: 568-572 [PMID: 8302794 DOI: 10.1097/00006676-199309000-00007]

88 **Rosendahl J**, Witt H, Szmola R, Bhatia E, Ozsvári B, Landt O, Schulz HU, Gress TM, Pfützer R, Löhr M, Kovacs P, Blüher M, Stumvoll M, Choudhuri G, Hegyi P, te Morsche RH, Drenth JP, Truninger K, Macek M, Puhl G, Witt U, Schmidt H, Büning C, Ockenga J, Kage A, Groneberg DA, Nickel R, Berg T, Wiedenmann B, Bödeker H, Keim V, Mössner J, Teich N, Sahin-Tóth M. Chymotrypsin C (CTRC) variants that diminish activity or secretion are associated with chronic pancreatitis. *Nat Genet* 2008; **40**: 78-82 [PMID: 18059268 DOI: 10.1038/ng.2007.44]

89 **Miyasaka K**, Ohta M, Takano S, Hayashi H, Higuchi S, Maruyama K, Tando Y, Nakamura T, Takata Y, Funakoshi A. Carboxylester lipase gene polymorphism as a risk of alcohol-induced pancreatitis. *Pancreas* 2005; **30**: e87-e91 [PMID: 15841033 DOI: 10.1097/01.mpa.0000160960.21580.mL]

90 **Ockenga J**, Vogel A, Teich N, Keim V, Manns MP, Strassburg CP. UDP glucuronosyltransferase (UGT1A7) gene polymorphisms increase the risk of chronic pancreatitis and pancreatic cancer. *Gastroenterology* 2003; **124**: 1802-1808 [PMID: 12806614 DOI: 10.1016/S0016-5085(03)00294-4]

91 **Witt H**, Luck W, Becker M, Böhmig M, Kage A, Truninger K, Ammann RW, O'Reilly D, Kingsnorth A, Schulz HU, Halangk W, Kielstein V, Knoefel WT, Teich N, Keim V. Mutation in the SPINK1 trypsin inhibitor gene, alcohol use, and chronic pancreatitis. *JAMA* 2001; **285**: 2716-2717 [PMID: 11386926 DOI: 10.1001/jama.285.21.2716-a]

92 **Schneider A**, Barmada MM, Slivka A, Martin JA, Whitcomb DC. Transforming growth factor-beta1, interleukin-10 and interferon-gamma cytokine polymorphisms in patients with hereditary, familial and sporadic chronic pancreatitis. *Pancreatology* 2004; **4**: 490-494 [PMID: 15316224 DOI: 10.1159/000080245]

93 **Perri F**, Piepoli A, Stanziale P, Merla A, Zelante L, Andriulli A. Mutation analysis of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, the cationic trypsinogen (PRSS1) gene, and the serine protease inhibitor, Kazal type 1 (SPINK1) gene in patients with alcoholic chronic pancreatitis. *Eur J Hum Genet* 2003; **11**: 687-692 [PMID: 12939655 DOI: 10.1038/sj.ejhg.5201035]

94 **Frenzer A**, Butler WJ, Norton ID, Wilson JS, Apte MV, Pirola RC, Ryan P, Roberts-Thomson IC. Polymorphism in alcohol-metabolizing enzymes, glutathione S-transferases and apolipoprotein E and susceptibility to alcohol-induced cirrhosis and chronic pancreatitis. *J Gastroenterol Hepatol* 2002; **17**: 177-182 [PMID: 11966948 DOI: 10.1046/j.1440-1746.2002.02670.x]

95 **Norton ID**, Apte MV, Dixson H, Trent RJ, Haber PS, Pirola RC, Wilson JS. Cystic fibrosis genotypes and alcoholic pancreatitis. *J Gastroenterol Hepatol* 1998; **13**: 496-499 [PMID: 9641647 DOI: 10.1111/j.1440-1746.1998.tb00675.x]

96 **Haber PS**, Wilson JS, McGarity BH, Hall W, Thomas MC, Pirola RC. Alpha 1 antitrypsin phenotypes and alcoholic pancreatitis. *Gut* 1991; **32**: 945-948 [PMID: 1885078 DOI: 10.1136/gut.32.8.945]

P- Reviewers: Bramhall SR, Dambrauskas Z, Falconi M, Sakata N
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Zhang DN

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7241

World J Gastroenterol 2013 November 14; 19(42): 7241-7246
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Morphological and functional evaluation of chronic pancreatitis with magnetic resonance imaging

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Received: June 12, 2013 Revised: July 13, 2013
Accepted: August 5, 2013
Published online: November 14, 2013

Abstract

Magnetic resonance imaging (MRI) techniques for assessment of morphology and function of the pancreas have been improved dramatically the recent years and MRI is very often used in diagnosing and follow-up of chronic pancreatitis (CP) patients. Standard MRI including fat-suppressed T1-weighted and T2-weighted imaging techniques reveal decreased signal and glandular atrophy of the pancreas in CP. In contrast-enhanced MRI of the pancreas in CP the pancreatic signal is usually reduced and delayed due to decreased perfusion as a result of chronic inflammation and fibrosis. Thus, morphological changes of the ductal system can be assessed by magnetic resonance cholangiopancreatography (MRCP). Furthermore, secretin-stimulated MRCP is a valuable technique to evaluate side branch pathology and the exocrine function of the pancreas and diffusion weighted imaging can be used to quantify both parenchymal fibrotic changes and the exocrine function of the pancreas. These standard and advanced MRI techniques are supplementary techniques to reveal morphological and functional changes of the pancreas in CP. Recently, spectroscopy has been used for assessment of metabolite concentrations *in-vivo* in different tissues and may have the potential to offer better tissue characterization of the pancreas. Hence, the purpose of the present review is to provide an update on standard and advanced MRI techniques of the pancreas in CP.

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Key words: Magnetic resonance; Chronic pancreatitis; Secretin; Diffusion weighted imaging; Exocrine pancreatic function

Core tip: Magnetic resonance imaging (MRI) techniques for assessment of morphology and function of the pancreas are often used in diagnosing and follow-up of chronic pancreatitis patients. The purpose of the present review is to provide an update on standard and advanced MRI techniques of the pancreas in chronic pancreatitis. In addition to standard MRI techniques, advanced MRI techniques including magnetic resonance cholangiopancreatography (MRCP), secretin-stimulated MRCP and diffusion weighted imaging can also provide important microstructural and functional information.

Hansen TM, Nilsson M, Gram M, Frøkjær JB. Morphological and functional evaluation of chronic pancreatitis with magnetic resonance imaging. *World J Gastroenterol* 2013; 19(42): 7241-7246 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7241.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7241

INTRODUCTION

The diagnosis and follow-up of chronic pancreatitis (CP) patients rely on both clinical and imaging information such as the Mayo Clinic diagnostic criteria[1]. Especially, the diagnosis of CP at an early stage is a clinical challenge. The development in imaging techniques, and especially magnetic resonance imaging (MRI), has dramatically improved the information on both morphology and function of the pancreas. In the Mayo diagnostic criteria for CP, MRI is now an accepted method for assessing ductal pathology and concrements[1].

A few decades ago, before the clinical introduction of cross-sectional imaging techniques, the imaging evaluation of CP was limited to plain radiography depicting calcifications. Traditionally, the ductal morphology has been assessed with endoscopic retrograde cholangiopancreatography (ERCP), which is based on intraductal contrast-enhancement with severity assessed using the Cambridge classification[2]. Today routine imaging modalities in the evaluation of CP typically include: Computed tomography (CT) with one or more contrast-enhancement phases, MRI with or without magnetic resonance cholangiopancreatography (MRCP) and ultrasound with a transabdominal or endoscopic approach. The advantage of MRI is the superior soft tissue visualization without radiation exposure. The main focus of imaging is typically to describe glandular atrophy and calcifications, duct pathology, pseudocysts and complications such as abscess formation and concomitant acute inflammation.

Recently, more advanced MRI methods have emerged which also provide important information on both tissue characteristics and pancreatic function.

The aim of this review is to provide an update on standard and advanced MRI techniques of the pancreas in CP.

STANDARD MRI

Parenchymal changes can be assessed in the early stage of CP by standard MRI typically including fat-suppressed T1-weighted images, T2-weighted images and gadolinium-enhanced imaging[3-6].

Fat-suppressed T1-weighted images show high signal intensity in normal pancreas and decreased signal intensity in CP due to loss of aqueous protein in the acini within the glandular elements of the pancreas caused by chronic inflammation and fibrosis[7-9]. Fat-suppression of T1-weighted images enhance the signal of the pancreas in relation to the surrounding retroperitoneal fat and the signal intensity can be compared to surrounding organs and tissue such as the spleen or muscles[9]. The biliary system and pancreatic ducts are enhanced on fat-suppressed T2-weighted images (Figure 1). The size of pancreas normally decreases with age but as acinar atrophy occurs more rapidly in CP, the pancreas diminishes segmentally or diffusely in the anteroposterior dimensions. The diameter can typically be assessed at the head, body and tail of the pancreas and compared with age-related normal values of the anteroposterior diameter[10].

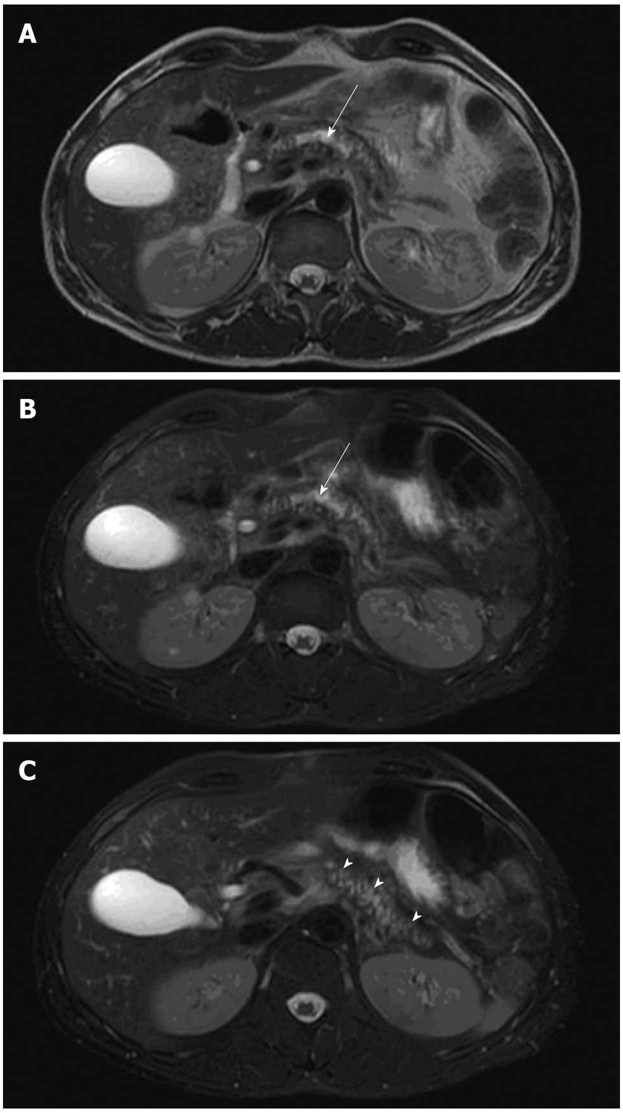

Figure 1 Pancreatic morphology. Axial T2-weighted magnetic resonance imaging views showing glandular atrophy (A), dilated irregular duct (B, arrow) and irregular side-branches (C, arrow heads) in a patient with chronic pancreatitis.

Gadolinium-enhanced imaging is used to investigate the perfusion of the pancreas during a series of contrast-enhanced images with repeated sequential scans. During the arterial phase of gadolinium infusion the normal pancreas shows pronounced enhancement due to high vascular perfusion and decreased signal during the venous phase[11]. The perfusion of the pancreas is decreased in patients with CP due to chronic inflammation and fibrosis resulting in reduced and delayed enhancement of the pancreatic signal[7,12].

Standard MRI is often used together with MRCP to assess both parenchymal and ductal changes of the pancreas. One important limitation of MRI lies in its inability to depict small parenchymal calcifications, which can easily be evaluated with CT.

MRI also has a role in diagnosing autoimmune pancreatitis (AIP) which can be a differential diagnosis to CP[13,14]. In diffuse AIP, MRCP may show a decreased di-

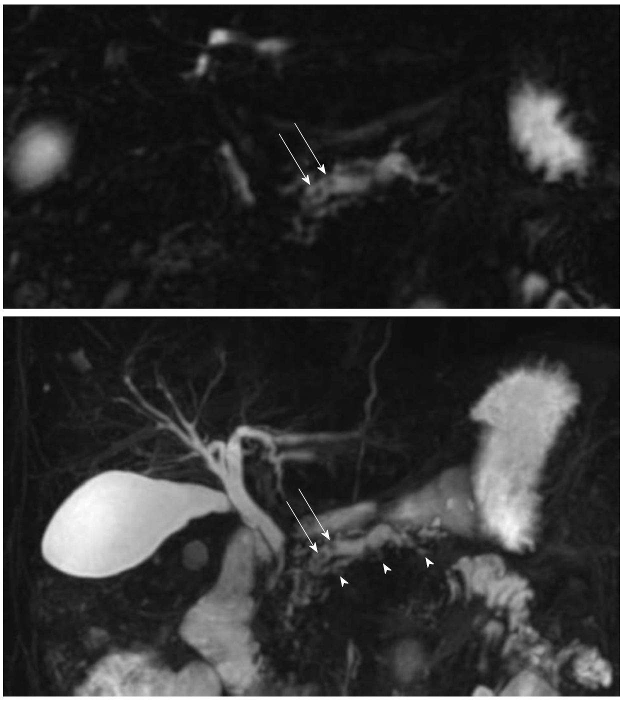

Figure 2 Magnetic resonance cholangiopancreatography. Upper figure displays a single coronal image and the lower figure a 3D view of the pancreato-biliary tree. In this chronic pancreatitis patient the dilated irregular main duct has irregular side branches (arrow heads) and contains multiple rounded filling defects (arrows).

ameter of the main pancreatic duct and can be accompanied by strictures and an irregular wall. However, in AIP the pancreas is typically with diffuse or localized enlargement with reduced signal on T1 and increased signal on T2-weighted images. A surrounding capsule with reduced signal in T2-weighted images can be seen[13,14].

MRCP

MRCP was first described in 1991, providing a non-invasive alternative to ERCP, which relies on the endoscopic injection of contrast fluid into the common bile duct[15]. By applying either a single-shot breath-hold technique or a free-breathing technique with respiratory triggering, MRCP can provide both 2D and 3D images[16,17]. Advances in scanner technology in the recent years allow faster image acquisition and better quality with more detailed images including 3D reconstructions. These advances benefit from a high signal-to-noise ratio. Additionally, the 3D free-breathing protocol makes it superior to 2D imaging in patients who are unable or unwilling to hold their breath for the duration of the scan[18].

MRCP relies on heavily T2-weighted pulse sequences, benefiting from the T2-weighted differences in relaxation time between fluid-filled compartments and adjacent soft tissue. Hence, the pancreato-biliary tree is displayed as high signal intensity and the pancreatic duct is clearly visualized in the normal pancreas. Accordingly, the MRCP technique is relevant in detecting pancreatic ductal dilatation, small filling defects (stones and protein plugs), strictures, irregularities of the main pancreatic duct, and irregularity (sacculation and/or ectasia) of side branches[6] (Figure 2). Furthermore, pseudocysts and other ductal congenital abnormalities and normal variants (such as pancreas divisum) can be visualized[19,20].

The Cambridge classification system of ductal dimensional changes has been modified for the MRCP technique in the following fashion: Cambridge 1 (normal pancreas): pancreatic ducts are normal; Cambridge 2 (equivocal pancreas): 1-2 side branches and main duct 2-4 mm; Cambridge 3 (mild disease): ≥ 3 side branches and main duct 2-4 mm; Cambridge 4 (moderate disease): ≥ 3 side branches and main duct > 4 mm; Cambridge 5 (marked disease): as traditional Cambridge classification system[2,6].

The acquisition of MRCP sequences following intravenous administration of secretin hormone allows a better visualization of subtle ductal changes especially in the early stage of CP[21].

SECRETIN-STIMULATED MRCP

The secretin hormone stimulates pancreatic duct cells to produce a large volume of watery bicarbonate-rich pancreatic juice, which is secreted into the ducts and duodenum. Typically, images are obtained before and frequently after secretin stimulation for a period. In the normal pancreas the effect starts almost immediately and peaks between 2-5 min and by 10 min the caliber of the duct should return to baseline[21]. In secretin-stimulated MRCP (s-MRCP) the ductal system including side branch pathology and filling defects are better visualized compared to traditional MRCP, and s-MRCP provides images comparable to ERCP with Cambridge classification[21-23]. Furthermore, the exocrine function can be evaluated with assessment of duodenal filling, changes in pancreatic duct caliber, change in anteroposterior diameter of the pancreas, and change in signal intensity ratio between pancreas and spleen on T1-weighted and arterial-venous enhancement ratios[10,23,24]. The s-MRCP findings in CP (reduced duodenal filling grade and reduced increase in pancreatic duct caliber) are comparable to the results of endoscopic pancreatic function testing (ePFT)[23]. Studies in CP by Manfredi *et al*[25] and Schneider *et al*[26] showed correlation between assessment of the pancreatic exocrine reserve by dynamic s-MRCP and exocrine function assessed by fecal elastase test and 13C-mixed chain triglyceride breath test. Wathle *et al*[27] found correlation between endoscopic aspiration-based bicarbonate test and s-MRCP findings in healthy controls. However, the use of s-MRCP has not yet been integrated as a part of the Mayo diagnostic criteria for CP and further evaluation of s-MRCP as a test for exocrine function in comparison to traditional tests is needed.

Furthermore, s-MRCP can be combined with diffusion weighted imaging (DWI) of the pancreas.

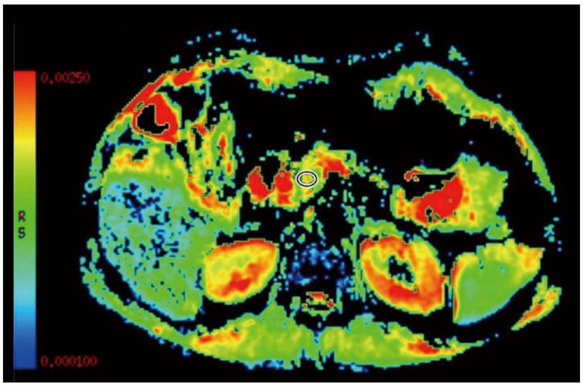

Figure 3 Diffusion weighted imaging. The apparent diffusion coefficient map of a chronic pancreatitis patient is shown with a measuring region of interest positioned in the pancreatic head to assess the degree of parenchymal fibrosis (red is high and blue low water diffusion).

DWI

DWI is an emerging technology to assess early parenchymal changes associated with CP and has shown results comparable to other existing methods such as MRCP and ePFT[9,28]. DWI assesses the random microscopic motion of water protons to obtain the apparent diffusion coefficient (ADC). The flow of water is less restricted in fluid-rich tissues, which will then be represented by a high ADC value. Water molecules interacting with cell membranes and macromolecules will be restricted thereby causing a reduction in the ADC value. A reduced amount of diffusible water is present in fibrotic tissue and in reduced pancreatic exocrine function[28]. Hence, presence of parenchymal fibrosis in CP causes diffusion restriction and results in lower ADC values[29,30] (Figure 3).

Furthermore, when used in combination with secretin stimulation, the ADC value increases both in normal pancreas and in CP as the secretion stimulation facilitates an increased mobility of water molecules and increased circulation in the pancreatic capillaries. Following secretin stimulation, the diffusion coefficients have either delayed or lower peak values in CP patients, indicating reduced exocrine function[29,31]. Also, patients in risk of CP (such as alcohol consumption, nicotine consumption, nutritional factors, hereditary factors, efferent duct obstructions, immunological factors and rare miscellaneous factors) generally exhibit a delayed peak in diffusion coefficients compared to controls[31].

This technique can be particularly useful in patients with early stage CP where atrophy and ductal pathology are subtle. Furthermore, the technique is useful in differentiating between pancreatic cysts, inflammatory cysts and cystic neoplasms and between pancreatic adenocarcinoma and normal pancreas due to different content of cellular elements[32]. Inan *et al*[33] calculated ADC values and ADC cyst-to-pancreas ratios and found significant lower values for abscesses, hydatid cysts and neoplastic cysts compared to values of simple cysts and pseudocysts. This technique may be useful in the diagnosis of intraductal papillary mucinous neoplasms (IPMN), which is often difficult since CP and IPMN may have overlapping imaging findings[13,34]. Patients with main duct IPMN can present with ductal dilatation and associated parenchymal atrophy, and patients with side-branch IPMN can present with cystic lesions often confused with pseudocysts. ADC values of the cystic lesions may be helpful in deciding the malignant potential of IPMN[34,35].

Table 1 Advantages of magnetic resonance imaging techniques

	MRI	MRCP	s-MRCP	DWI
Loss of aqueous protein	Yes	No	No	No
Glandular atrophy	Yes	No	No	No
Perfusion	Yes	No	No	No
Calcification[1]	No	No	No	No
Pancreatic ductal dilation	No	Yes	No	No
Filling defects	No	Yes	Yes	No
Strictures	No	Yes	Yes	No
Irregularities	No	Yes	Yes	No
Pseudocysts	Yes	Yes	Yes	No
Side branch pathology	No	Yes	Yes	No
Exocrine function	No	No	Yes	Yes
Parenchymal fibrosis	No	No	No	Yes

Advantages of the different magnetic resonance imaging (MRI) techniques in diagnosing chronic pancreatitis. [1]A disadvantage of MRI is the inability to detect calcifications. This can be achieved through computed tomography scans. DWI: Diffusion weighted imaging; MRCP: Magnetic resonance cholangiopancreatography; s-MRCP: Secretin-stimulated MRCP.

NEW TECHNIQUES

Recently, other advanced MRI techniques have become more interesting as the development within the MR hardware and software is expanding and allows increased signal-to-noise ratios, shorter scan time and breath-hold imaging.

MR spectroscopy with non-invasive *in-vivo* assessment of metabolite concentrations has been applied in a variety of different tissues (*e.g.*, brain, prostate, breast and liver). Hence, spectroscopy of the pancreas has the potential to offer a more accurate tissue characterization. Due to methodological challenges, the pancreas has only been studied to a very limited degree with spectroscopy. Despite of this, Su *et al*[36] characterized the normal pancreas at 3T and identified metabolites such as lipid, choline and cholesterol. Cho *et al*[37] used MR spectroscopy to distinguish between patients with chronic focal pancreatitis and patients with pancreatic carcinoma and found less lipid in pancreatitis than in pancreatic carcinoma. Furthermore, other studies also detected differences between normal pancreatic tissue and carcinoma tissue with alterations in lipid, choline and fatty acids[38,39]. However, to the best of our knowledge, this technique has not yet been applied in the characterization of CP patients.

CONCLUSION

This review provides an update on standard and advanced MRI of the pancreas in CP. Table 1 summarizes the capability of the different MRI techniques to display the different aspects of pancreatic changes and dysfunction in CP. Depending on local practice, scanner configuration and radiological experience, it should be possible to construct or customize individual MR protocols including the (or some of the) techniques reviewed in this paper to get the best possible morphological and functional information in CP.

REFERENCES

1 **Layer P**, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology* 1994; **107**: 1481-1487 [PMID: 7926511]

2 **Sarner M**, Cotton PB. Classification of pancreatitis. *Gut* 1984; **25**: 756-759 [PMID: 6735257 DOI: 10.1136/gut.25.7.756]

3 **Semelka RC**, Kroeker MA, Shoenut JP, Kroeker R, Yaffe CS, Micflikier AB. Pancreatic disease: prospective comparison of CT, ERCP, and 1.5-T MR imaging with dynamic gadolinium enhancement and fat suppression. *Radiology* 1991; **181**: 785-791 [PMID: 1947098]

4 **Semelka RC**, Shoenut JP, Kroeker MA, Micflikier AB. Chronic pancreatitis: MR imaging features before and after administration of gadopentetate dimeglumine. *J Magn Reson Imaging* 1993; **3**: 79-82 [PMID: 8428105 DOI: 10.1002/jmri.1880030114]

5 **Sica GT**, Miller FH, Rodriguez G, McTavish J, Banks PA. Magnetic resonance imaging in patients with pancreatitis: evaluation of signal intensity and enhancement changes. *J Magn Reson Imaging* 2002; **15**: 275-284 [PMID: 11891972 DOI: 10.1002/jmri.10066]

6 **Choueiri NE**, Balci NC, Alkaade S, Burton FR. Advanced imaging of chronic pancreatitis. *Curr Gastroenterol Rep* 2010; **12**: 114-120 [PMID: 20424983 DOI: 10.1007/s11894-010-0093-4]

7 **Ly JN**, Miller FH. MR imaging of the pancreas: a practical approach. *Radiol Clin North Am* 2002; **40**: 1289-1306 [PMID: 12479712 DOI: 10.1016/S0033-8389(02)00056-8]

8 **Ito K**, Koike S, Matsunaga N. MR imaging of pancreatic diseases. *Eur J Radiol* 2001; **38**: 78-93 [PMID: 11335090 DOI: 10.1016/S0720-048X(01)00293-5]

9 **Balcı C**. MRI assessment of chronic pancreatitis. *Diagn Interv Radiol* 2011; **17**: 249-254 [PMID: 20945291 DOI: 10.4261/1305-3825.DIR.3889-10.0]

10 **Balci NC**, Alkaade S, Magas L, Momtahen AJ, Burton FR. Suspected chronic pancreatitis with normal MRCP: findings on MRI in correlation with secretin MRCP. *J Magn Reson Imaging* 2008; **27**: 125-131 [PMID: 18058927 DOI: 10.1002/jmri.21241]

11 **Balci NC**, Alkaade S, Akduman IE, Bilgin M, Murdock CP, Burton FR. Serial contrast-enhanced MRI of the pancreas: correlation with secretin-stimulated endoscopic pancreatic function test. *Acad Radiol* 2006; **13**: 1367-1372 [PMID: 17070454 DOI: 10.1016/j.acra.2006.08.008]

12 **Coenegrachts K**, Van Steenbergen W, De Keyzer F, Vanbeckevoort D, Bielen D, Chen F, Dockx S, Maes F, Bosmans H. Dynamic contrast-enhanced MRI of the pancreas: initial results in healthy volunteers and patients with chronic pancreatitis. *J Magn Reson Imaging* 2004; **20**: 990-997 [PMID: 15558558 DOI: 10.1002/jmri.20212]

13 **Perez-Johnston R**, Sainani NI, Sahani DV. Imaging of chronic pancreatitis (including groove and autoimmune pancreatitis). *Radiol Clin North Am* 2012; **50**: 447-466 [PMID: 22560691 DOI: 10.1016/j.rcl.2012.03.005]

14 **Detlefsen S**, Löhr JM, Drewes AM, Frøkjær JB, Klöppel G. Current concepts in the diagnosis and treatment of type 1 and type 2 autoimmune pancreatitis. *Recent Pat Inflamm Allergy Drug Discov* 2011; **5**: 136-149 [PMID: 21453268 DOI: 10.2174/187221311795399228]

15 **Wallner BK**, Schumacher KA, Weidenmaier W, Friedrich JM. Dilated biliary tract: evaluation with MR cholangiography with a T2-weighted contrast-enhanced fast sequence. *Radiology* 1991; **181**: 805-808 [PMID: 1947101]

16 **Takehara Y**, Ichijo K, Tooyama N, Kodaira N, Yamamoto H, Tatami M, Saito M, Watahiki H, Takahashi M. Breath-hold MR cholangiopancreatography with a long-echo-train fast spin-echo sequence and a surface coil in chronic pancreatitis. *Radiology* 1994; **192**: 73-78 [PMID: 8208969]

17 **Barish MA**, Yucel EK, Soto JA, Chuttani R, Ferrucci JT. MR cholangiopancreatography: efficacy of three-dimensional turbo spin-echo technique. *AJR Am J Roentgenol* 1995; **165**: 295-300 [PMID: 7618543 DOI: 10.2214/ajr.165.2.7618543]

18 **Kim JH**, Hong SS, Eun HW, Han JK, Choi BI. Clinical usefulness of free-breathing navigator-triggered 3D MRCP in non-cooperative patients: comparison with conventional breath-hold 2D MRCP. *Eur J Radiol* 2012; **81**: e513-e518 [PMID: 21700409 DOI: 10.1016/j.ejrad.2011.06.004]

19 **Kamisawa T**, Tu Y, Egawa N, Tsuruta K, Okamoto A, Kamata N. MRCP of congenital pancreaticobiliary malformation. *Abdom Imaging* 2007; **32**: 129-133 [PMID: 16680507 DOI: 10.1007/s00261-006-9005-3]

20 **Yu J**, Turner MA, Fulcher AS, Halvorsen RA. Congenital anomalies and normal variants of the pancreaticobiliary tract and the pancreas in adults: part 2, Pancreatic duct and pancreas. *AJR Am J Roentgenol* 2006; **187**: 1544-1553 [PMID: 17114549 DOI: 10.2214/AJR.05.0774]

21 **Griffin N**, Charles-Edwards G, Grant LA. Magnetic resonance cholangiopancreatography: the ABC of MRCP. *Insights Imaging* 2012; **3**: 11-21 [PMID: 22695995 DOI: 10.1007/s13244-011-0129-9]

22 **Lee NJ**, Kim KW, Kim TK, Kim MH, Kim SY, Park MS, Kim AY, Ha HK, Kim PN, Lee MG. Secretin-stimulated MRCP. *Abdom Imaging* 2006; **31**: 575-581 [PMID: 16465582 DOI: 10.1007/s00261-005-0118-x]

23 **Balci NC**, Smith A, Momtahen AJ, Alkaade S, Fattahi R, Tariq S, Burton F. MRI and S-MRCP findings in patients with suspected chronic pancreatitis: correlation with endoscopic pancreatic function testing (ePFT). *J Magn Reson Imaging* 2010; **31**: 601-606 [PMID: 20187202 DOI: 10.1002/jmri.22085]

24 **Nøjgaard C**, Olesen SS, Frøkjaer JB, Drewes AM. Update of exocrine functional diagnostics in chronic pancreatitis. *Clin Physiol Funct Imaging* 2013; **33**: 167-172 [PMID: 23522009 DOI: 10.1111/cpf.12011]

25 **Manfredi R**, Perandini S, Mantovani W, Frulloni L, Faccioli N, Pozzi Mucelli R. Quantitative MRCP assessment of pancreatic exocrine reserve and its correlation with faecal elastase-1 in patients with chronic pancreatitis. *Radiol Med* 2012; **117**: 282-292 [PMID: 22231574 DOI: 10.1007/s11547-011-0774-6]

26 **Schneider AR**, Hammerstingl R, Heller M, Povse N, Murzynski L, Vogl TJ, Caspary WF, Stein J. Does secretin-stimulated MRCP predict exocrine pancreatic insufficiency?: A comparison with noninvasive exocrine pancreatic function tests. *J Clin Gastroenterol* 2006; **40**: 851-855 [PMID: 17016144 DOI: 10.1097/01.mcg.0000225652.00308.a2]

27 **Wathle GK**, Tjora E, Ersland L, Dimcevski G, Salvesen OO, Molven A, Njølstad PR, Haldorsen IS. Assessment of exocrine pancreatic function by secretin-stimulated magnetic resonance cholangiopancreaticography and diffusion-weighted imaging in healthy controls. *J Magn Reson Imaging* 2013; Epub ahead of print [PMID: 23649590 DOI: 10.1002/jmri.24167]

28 **Balci NC**, Momtahen AJ, Akduman EI, Alkaade S, Bilgin M, Burton FR. Diffusion-weighted MRI of the pancreas: correlation with secretin endoscopic pancreatic function test (ePFT). *Acad Radiol* 2008; **15**: 1264-1268 [PMID: 18790398 DOI: 10.1016/j.acra.2008.05.002]

29 **Akisik MF**, Aisen AM, Sandrasegaran K, Jennings SG, Lin C, Sherman S, Lin JA, Rydberg M. Assessment of chronic pancreatitis: utility of diffusion-weighted MR imaging with

secretin enhancement. *Radiology* 2009; **250**: 103-109 [PMID: 19001148 DOI: 10.1148/radiol.2493080160]

30 **Frøkjær JB**, Olesen SS, Drewes AM. Fibrosis, atrophy, and ductal pathology in chronic pancreatitis are associated with pancreatic function but independent of symptoms. *Pancreas* 2013; **42**: 1182-1187 [PMID: 24048457]

31 **Erturk SM**, Ichikawa T, Motosugi U, Sou H, Araki T. Diffusion-weighted MR imaging in the evaluation of pancreatic exocrine function before and after secretin stimulation. *Am J Gastroenterol* 2006; **101**: 133-136 [PMID: 16405545 DOI: 10.1111/j.1572-0241.2006.00406.x]

32 **Balci NC**, Perman WH, Saglam S, Akisik F, Fattahi R, Bilgin M. Diffusion-weighted magnetic resonance imaging of the pancreas. *Top Magn Reson Imaging* 2009; **20**: 43-47 [PMID: 19687725 DOI: 10.1097/RMR.0b013e3181b48667]

33 **Inan N**, Arslan A, Akansel G, Anik Y, Demirci A. Diffusion-weighted imaging in the differential diagnosis of cystic lesions of the pancreas. *AJR Am J Roentgenol* 2008; **191**: 1115-1121 [PMID: 18806153 DOI: 10.2214/AJR.07.3754]

34 **Sandrasegaran K**, Akisik FM, Patel AA, Rydberg M, Cramer HM, Agaram NP, Schmidt CM. Diffusion-weighted imaging in characterization of cystic pancreatic lesions. *Clin Radiol* 2011; **66**: 808-814 [PMID: 21601184 DOI: 10.1016/j.crad.2011.01.016]

35 **Kang KM**, Lee JM, Shin CI, Baek JH, Kim SH, Yoon JH, Han JK, Choi BI. Added value of diffusion-weighted imaging to MR cholangiopancreatography with unenhanced MR imaging for predicting malignancy or invasiveness of intraductal papillary mucinous neoplasm of the pancreas. *J Magn Reson Imaging* 2013; Epub ahead of print [PMID: 23390008 DOI: 10.1002/jmri.24022]

36 **Su TH**, Jin EH, Shen H, Zhang Y, He W. In vivo proton MRS of normal pancreas metabolites during breath-holding and free-breathing. *Clin Radiol* 2012; **67**: 633-637 [PMID: 22316597 DOI: 10.1016/j.crad.2011.05.018]

37 **Cho SG**, Lee DH, Lee KY, Ji H, Lee KH, Ros PR, Suh CH. Differentiation of chronic focal pancreatitis from pancreatic carcinoma by in vivo proton magnetic resonance spectroscopy. *J Comput Assist Tomogr* 2005; **29**: 163-169 [PMID: 15772531 DOI: 10.1097/01.rct.0000153956.33296.b5]

38 **Yao X**, Zeng M, Wang H, Fei S, Rao S, Ji Y. Metabolite detection of pancreatic carcinoma by in vivo proton MR spectroscopy at 3T: initial results. *Radiol Med* 2012; **117**: 780-788 [PMID: 22095426 DOI: 10.1007/s11547-011-0757-7]

39 **Ma X**, Zhao X, Ouyang H, Sun F, Zhang H, Zhou C, Shen H. The metabolic features of normal pancreas and pancreatic adenocarcinoma: preliminary result of in vivo proton magnetic resonance spectroscopy at 3.0 T. *J Comput Assist Tomogr* 2011; **35**: 539-543 [PMID: 21926845 DOI: 10.1097/RCT.0b013e318227a545]

P- Reviewers: Abraham P, Hard PD, Kamisawa T, Pezzilli R
S- Editor: Gou SX **L- Editor**: A **E- Editor**: Zhang DN

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7247

World J Gastroenterol 2013 November 14; 19(42): 7247-7257
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

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TOPIC HIGHLIGHT

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Ultrasonography in diagnosing chronic pancreatitis: New aspects

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Received: June 19, 2013 **Revised:** September 6, 2013
Accepted: September 16, 2013
Published online: November 14, 2013

Abstract

The course and outcome is poor for most patients with pancreatic diseases. Advances in pancreatic imaging are important in the detection of pancreatic diseases at early stages. Ultrasonography as a diagnostic tool has made, virtually speaking a technical revolution in medical imaging in the new millennium. It has not only become the preferred method for first line imaging, but also, increasingly to clarify the interpretation of other imaging modalities to obtain efficient clinical decision. We review ultrasonography modalities, focusing on advanced pancreatic imaging and its potential to substantially improve diagnosis of pancreatic diseases at earlier stages. In the first section, we describe scanning techniques and examination protocols. Their consequences for image quality and the ability to obtain complete and detailed visualization of the pancreas are discussed. In the second section we outline ultrasonographic characteristics of pancreatic diseases with emphasis on chronic pancreatitis. Finally, new developments in ultrasonography of the pancreas such as contrast enhanced ultrasound and elastography are enlightened.

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Key words: Ultrasonography; Pancreas; Chronic pancreatitis; Transabdominal ultrasound; Medical imaging technique; Contrast enhanced ultrasonography; Elastography; Strain imaging

Core tip: Pancreatic diseases include acute and chronic inflammatory diseases and neoplastic tumors. It is a clinical challenge to diagnose these patients at an early stage because biochemical and imaging signs may be unspecific and are only evident at an advanced stage of the disease. Advances in pancreatic imaging are important for early detection of pancreatic diseases. Ultrasonography, as a diagnostic tool, has undergone major technical improvements over the last decade. It still is the preferred method for first line imaging, as well as it is increasingly used to clarify findings by others imaging modalities to support decision making.

Dimcevski G, Erchinger FG, Havre R, Gilja OH. Ultrasonography in diagnosing chronic pancreatitis: New aspects. *World J Gastroenterol* 2013; 19(42): 7247-7257 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7247.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7247

INTRODUCTION

Transabdominal ultrasonography still is the most used first line imaging modality in the diagnostic workup of abdominal diseases. Ultrasonography is, noninvasive, widely available, inexpensive, without side effects and easy to perform on a daily basis, if needed. As a "real-time" imaging modality, transabdominal ultrasonography gives first a broad overview and then can localize the "region of interest" to perform detailed evaluation and eventually determine the cause of the disease. It can also reduce the use of computed tomography, magnetic resonance pancreatography, endoscopic ultrasonography or other diagnostic methods, which are personnel-intensive and thereby costly[1,2].

Ultrasonography (US) of the pancreas is challenging, given its retroperitoneal location with overlying structures and relatively small size. The quality and thereby the clinical usefulness of the pancreatic ultrasound imaging has rapidly advanced along with the technological progress. Early works in the seventies and eighteens describe evidence of pancreatic structural changes seen by transabdominal ultrasonography of approximately two-thirds in the diagnosed chronic pancreatitis (CP) cases[3-7]. Concerning pancreatic malignancy, most early works describe, somewhat surprisingly, pathological findings in the majority of the cases, but the distinction between malignancy and chronic pancreatitis could not be achieved[3-7]. With modern, high-end scanners, experienced physicians could achieve complete imaging of the pancreas in 90% in the nineties[6]. The current sensitivity and specificity of transabdominal ultrasonography in the diagnosis of pancreatic diseases, the ability to differentiate between acute and chronic inflammation and premalignant or malignant lesions is not yet determined. Today we have real-time, high-resolution imaging, where the spatial resolution is in line with the best image quality computed tomography (CT) or magnet resonance imaging (MRI) can offer. Furthermore, the temporal resolution far exceeds that of CT, positron emission tomography and MRI, which is particularly relevant for contrast-enhanced ultrasonography (CEUS). Moreover, the image quality and especially the amount of information are evolving along with the technological progression and introduction of new modalities such as of CEUS and elastography[8,9].

Figure 1 shows a standard B-mode image of the pancreas, liver and surrounding vessels with a 1-5 and 12-15 MHz transducer. A clear overview of the gland and surrounding structures can be obtained and by changing the frequencies for any depth, focus on a smaller area of interest can be achieved.

We shortly review the current and forthcoming technical modalities and methods in transabdominal ultrasonography and endoscopic ultrasonography (EUS) of the pancreas with emphasis on CP. In our opinion, transabdominal ultrasonography has an important place in imaging of the pancreas, alongside CT, MRI and EUS.

ULTRASOUND EXAMINATION

Ultrasonographic imaging of the pancreas can be difficult given its retroperitoneal location, variety in appearance among individuals and body habitus. Overlying bowel gas and obesity are the most frequent limitations in transcutaneous scanning of the pancreas[10-12].

Due to the food related production of intestinal gas reflecting the ultrasound beams, every examination should be performed fasting. US examination of the pancreas includes transverse, longitudinal and angled oblique scans. Successful visualization can often be achieved by manipulations with the transducer and is directly linked to the skill and persistence of the examiner. By applying graded compression by the transducer, bowel gas can be moved away, and all the portions of the pancreatic gland; head, neck, body and tail can often be visualized. Further improvement can also be obtained by drinking two glasses of water/juice, thereby using the fluid-filled stomach as an acoustic window. Other manipulations such as; changing the patient's position to stand or sit; let the patient turn from the supine position to oblique position or change/stop the breathing circle; let the patient "blow up the belly", can also improve visualization of the pancreas[13].

The pancreatic tail can sometimes be difficult to access in transversal scan. Figure 2 shows trans-splenic access, where the tail of the pancreas (cauda pancreatis) is scanned from the left lateral side using the spleen as acoustic window.

In a US examination of the pancreas the echotexture, the size of the gland including the main pancreatic duct (MPD), and anatomical landmarks of the pancreas should be evaluated. The echotexture in a normal pancreas is isoechogenic or hyperechogenic compared to the healthy liver[14]. Frequently, the echogenicity of pancreas is increasing with age. Orientational antero-posterior dimensions of the pancreas are: the head (2.5 cm), body (1.5 cm), tail (3.5 cm) and the pancreatic duct (< 2.5 mm). Fatty replacement (lipomatosis) of the pancreatic gland and decrease in size is common with increasing age but can also be found in patients with cystic fibrosis, CP, some types of diabetes and other diseases[15-17].

The surrounding vascular, ductal and abdominal organ landmarks are the portal vein, splenic vein, confluens with the mesenteric vein, vena cava, aorta, superior mesenteric artery, common hepatic artery, splenic artery, common bile duct, duodenum, stomach and the liver.

MODALITIES IN ULTRASONOGRAPHY

Grayscale B-mode ultrasound

Grayscale B-mode ultrasound of the pancreas is the most used imaging modality. Complete evaluation of organ size, borders, echo structure, surrounding vessels, and pancreatic ducts can be obtained swiftly. These parameters are often sufficient to diagnose many diseases of the pancreas and patients can be managed accordingly.

Tissue harmonic imaging

Tissue harmonic imaging (THI) or second harmonic imaging overcomes several of the B-mode limits. By receiving harmonic overtones instead of the emitted US-frequencies, the lateral delineation is sharpened and

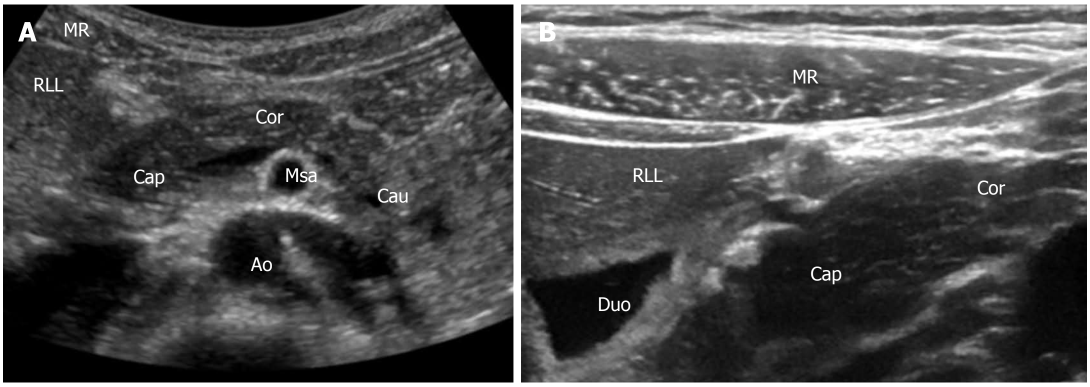

Figure 1 Pancreas and the surrounding anatomical landmarks. A: B-mode image (1-5 MHz); B: B-mode image with a 12-15 MHz transducer. Details shown with high resolution. MR: Musculus rectus abdominis; RLL: Right liver lobe; Cap: Caput pancreatis; Cor: Corpus pancreatis; Cau: Cauda pancreatis; Msa: Superior mesenteric artery; Duo: Duodenum; Ao: Aorta.

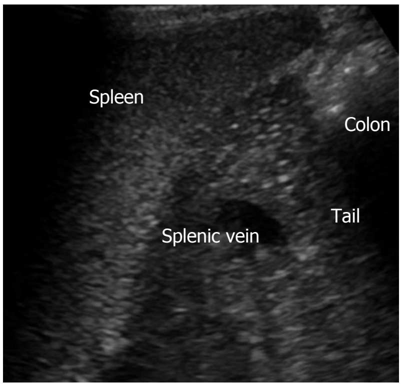

Figure 2 Left lateral side scan shows the pancreatic tail (cauda) using the spleen as acoustic window.

reverberation artifacts are reduced[18]. The image quality is improved by better discrimination between liquid and solid structures increasing the spatial and contrast resolution, making millimeter sized structures detectable. Thus, ultrasonography with THI may have better resolution than CT and MRI, in the absence of extreme obesity or a large amount of intestinal gas, which may temporarily mask the pancreas[19-21]. There are only a few disadvantages of THI; reduction of frame rate, reduced penetration depth, motion artifacts and only marginal improvement of image quality in the near sound field. This modality is normally used with CEUS, which is probably the most important application of this modality[22,23].

Doppler imaging

The Doppler effect in ultrasound is the change in US frequency of reflected wave from an object moving relative to the ultrasound probe, adding the option to show and record blood flow direction and velocity from vessels in ultrasound imaging.

Several modalities in conventional Doppler ultrasonography exist[24]. Combining B-mode gray scale and color-Doppler ultrasonography, overall accuracy rises substantially. By international convention, Doppler color is coded in such a way that red color expresses flow towards the ultrasound transducer, while blue color expresses flow away from the transducer. The recent technological progress, in particular increased color-Doppler sensitivity is contributing to diagnosing and staging of pancreatic diseases. Doppler interrogation of the gland may show the outlines of the organ more precisely, since the pancreas is surrounded by vessels. The normal intrapancreatic vessels are small and difficult to show in conventional Doppler imaging. Color-Doppler shows flows in normal surrounding vessels and abnormal vascularity, such as in tumors with high vessel infiltration or tumor infiltration in vessels[25]. This method can also discriminate between cystic avascular processes without blood flow and aneurisms. Very small pancreatic calcifications which can be hard to distinguish in the irregular parenchymal pancreatic tissue in CP can be identified by the presence of twinkling artifacts. Modern high- end scanners can distinguish between inflammation (high flow) and infarction without flow[26].

METHODS IN ULTRASONOGRAPHY

Panoramic imaging

In a conventional ultrasound field of view, only parts of the pancreas are visible in one image. With the panorama technique, based on real time image reconstruction the whole pancreas can be displayed in one image, allowing the creation of images similar to those in CT and MRI. However, the usefulness of these computed images regarding the evaluation of pancreatic disorders is not well documented.

Compound imaging

Compound imaging obtains multiple coplanar images from different angles using computed beam steering. Multiple views are integrated into a single compound image with improved tissue definition, where signals from real structures are intensified and the artifacts are suppressed[14,18,19]. Organs appear smoother with clear contours. The MPD and vessels are clearly accentuated. As these images are generated from several angles, classi-

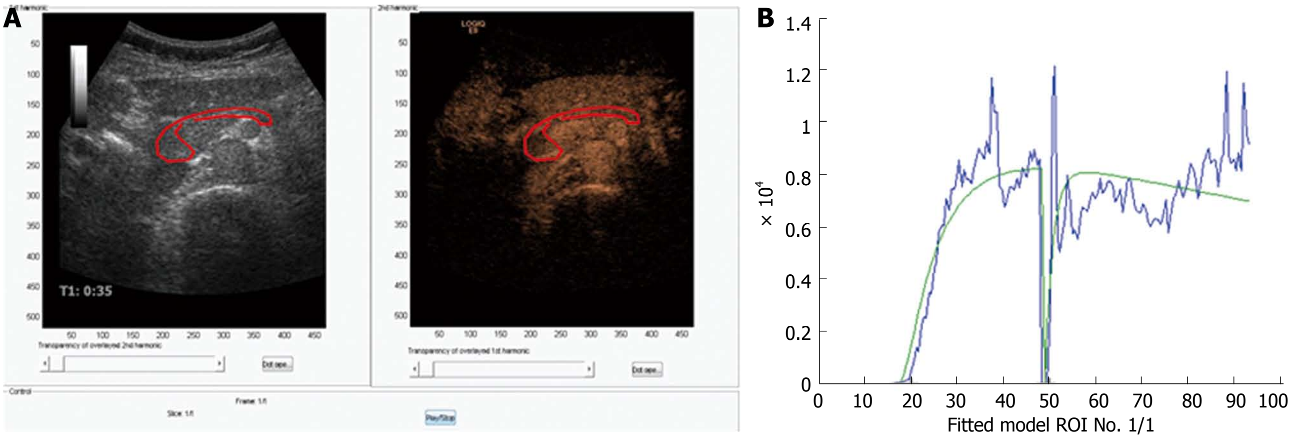

Figure 3 Perfusion analysis of the pancreas. A: Dual view of contrast-enhanced ultrasonography examination of the pancreas in a healthy volunteer. 1.5 mL bolus of Sonovue was given as a bolus and after approximately 45 s the area of interest was exposed to high MI ultrasound bursting the bubbles in the imaging plane; B: A motion correcting analyzing software was used (DCE-US, http://www.isibrno.cz/perfusion/). A region of interest have been drawn including the head and body of the pancreas (unpublished data).

cal signs in transabdominal ultrasonography such as cast shadows behind pancreatic calcifications or ultrasound enhancement behind pancreatic cysts are attenuated[27].

Contrast-enhanced ultrasonography

The development of microbubble contrast agents enables the display of the vasculature, down to parenchymal microvasculature. With this technique enhancement patterns of lesions can be studied in real time in a similar way as contrast-enhanced CT or contrast-enhanced MRI, under full control of the operator[28-31]. Ultrasound contrast agents are confined to the blood pool where CT or MRI contrast agents are rapidly cleared into the extravascular space. As the contrast bubbles, approximately the size of a red blood cell, are gas-filled, they create strong acoustic reflectors within the fluid filled blood pool. An advantage of CEUS is the ability to study the dynamics of lesions in real time. The excellent tolerance and safety profiles allow repeated administrations in the same session if needed. The elimination of the contrast agent is by breathing, independent of liver and kidney function. Today, CEUS is increasingly incorporated in clinical use, alongside with traditional US, in liver lesions, which is by the far the most common use, but also in the kidneys, in vesico-ureteric reflux, in trauma and in the cerebral circulation[28].

Transabdominal ultrasonography with CEUS in the pancreas has probably near the same potential, and has in some few centers been established in diagnostic routine[14,31]. The most common application is to define pancreatic lesions, in most cases already known lesions, previously seen on CT/MRI or seen in the initial US examination. Several studies show that transabdominal CEUS can be used to differentiate malignancy and CP[32-36]. The time window in a pancreatic CEUS examination is notably shorter than in the well-known liver CEUS study. This is due to the entirely arterial blood supply of the pancreas. The enhancement of the pancreas begins almost simultaneously with the aortic enhancement and reaches its peak between 15 and 20 s after injection of the ultrasound contrast agent. After a noticeable parenchymal enhancement in the arterial early phase, there is a washout of contrast medium with gradual loss of echogenicity during the late phase for approximately 120 s[36,37].

New technologies may be able to discriminate between normal pancreatic perfusion and pathological in parenchymal pancreatic diseases or different tumor perfusion patterns that can be visualized using the small microbubbles of ultrasound contrast agents[38]. Time-intensity curves expressing arrival time of the microbubbles, time to peak or other parameters have so far not been able to show significant difference in focal masses in patients with CP and carcinomas[34]. The first generation analyzing software did not incorporate motion correction and that can be one of the reasons for the insensitivity of this method, since the pancreas moves in both planes, thereby moving the region of interest out of plane. New versions of more sophisticated analyzing software are under development. Figure 3 shows an example of a CEUS examination of the pancreas with a motion tracked area of interest.

In the near future, microbubbles may be an important part of the therapeutic armaments[39]. Studies have been undertaken to investigate the ability and efficacy of sonoporation in a clinical setting to increase the overall survival in patients with pancreatic adenocarcinoma. In a pilot study, numbers of treatment cycles with gemcitabine were increased from an average of 9 to 16 cycles. In two out of five patients treated, the maximum tumor diameter was temporally decreased to 80% ± 5% and permanently to 70% ± 5% of their original size, whilst the other patients showed reduced growth. This study demonstrated that it is possible to combine ultrasound, microbubbles, and chemotherapy in a clinical setting prolonging the quality of life in patients with pancreatic adenocarcinoma when comparing to chemotherapy alone. Figure 4 shows an experimental microbubble sonopora-

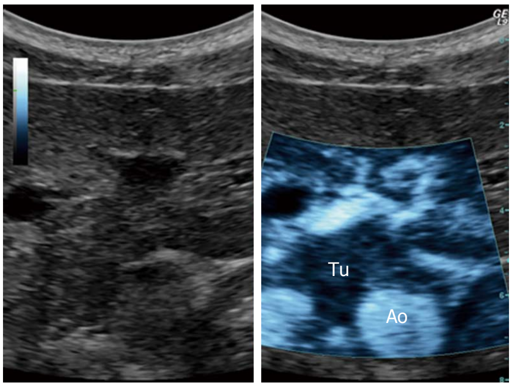

Figure 4 Ultrasound targeted treatment of pancreatic cancer using combined microbubbles and a chemotherapeutic. Left: B-mode frame. Right: The main tumor with spicules, clearly demarked, using the sonoporation settings. Tu: Tumor; Ao: Aorta.

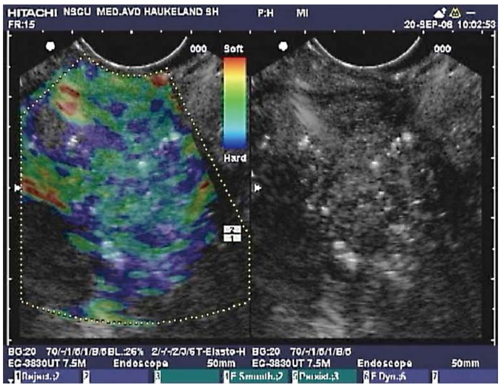

Figure 5 Endoscopic ultrasound with elastography of chronic pancreatitis. Endoscopic ultrasonography B-mode sonogram (right) and an elastogram superimposed a sonogram (left). In this image of the pancreatic head, hyperechoic foci and strands are seen in the parenchyma, as well as inhomogeneous echogenecity, which are signs of chronic pancreatitis. The elastogram shows predominantly a blue, indicating harder tissue, and green representing intermediate hardness in a honeycomb pattern over the pancreatic tissue.

tion therapy setup targeting a pancreatic adenocarcinoma, using a clinical ultrasound scanner[39].

ENDOSCOPIC ULTRASONOGRAPHY IN THE WORKUP OF CP

Even if transcutaneous US may image the pancreas in many cases, the limitations of obesity, reverberations and bowel gas may not always be eliminated. EUS has the advantage over trans-cutaneous US examinations that the US probe can be placed close to the organ of interest allowing the use of high frequency ultrasound (5-12.5 MHz). This may allow the imaging of small alterations in the pancreatic duct and parenchyma. The findings are usually grouped as ductal changes or parenchymal changes. The classic features include: ductal calcifications, hyperechoic duct walls, duct-caliber dilatation, visible side ducts. Parenchymal changes include: lobulation with or without honeycombing, parenchymal hyperecohic areas with or without shadowing, echogenic strands and cysts. The problem is that some of these features also are found in healthy subjects, and increasing with age. A study compared EUS and an endoscopic pancreatic function test (ePFT) sampling the peak bicarbonate concentration after secretin stimulation. EUS showed a good correlation with fibrosis. The sensitivity of EUS and ePFT had similar sensitivity for diagnosing CP (0.84%-0.86%), but EUS was more specific (100% *vs* 67%). By combining the two methods, however, the sensitivity reached 100%[40]. The Rosemont classification is a set of EUS-based criteria for the diagnosis of CP. These criteria are based on detailed evaluation on pancreatic parenchymal and ducts. Both parenchymal and ductal features are divided in major and minor criteria[41]. Major criteria for CP are; hyperechoic foci with shadowing, lobularity with honeycombing and MPD calculi. Minor criteria for CP; cysts, dilated ducts $\geqslant$ 3.5 mm, irregular pancreatic duct contour, dilated side branches $\geqslant$ 1 mm, hyperechoic duct wall, strands, non shadowing hyperechoic foci, and lobularity with noncontiguous lobules. The Rosemont criteria, using a combination of major and/or minor criteria, categorize the patient into four groups: consistent, suggestive, indeterminate of CP or normal.

NEW IMAGING METHODS FOR EUS IN CP-EUS ELASTOGRAPHY

Elasticity imaging of pancreatic tissue may help the examiner distinguish between harder and softer tissue. Elasticity imaging is currently an option in ultrasonography. Two main methodologies are commercially available. Strain imaging provides a qualitative strain map, frequently in a colored pattern superimposed on a B-mode echogram, visualizing the local strain as a result of endogeneous movements or by an acoustic pulse. Alternatively, a shear wave method is used, providing quantitative elasticity information based on the travelling speed of shear waves. The energy for these shear waves is deposited in the tissue by an acoustic pulse. In some systems both deformation based images and local shear-wave quantification are combined.

Up to now, only strain based imaging systems have been available in combination with flexible echo-endoscopes. In Figure 5 an EUS image of a pancreas with CP is imaged with Real-Time Elastography. In Figure 6 a reactive hilar lymph node in a patient with CP is imaged as green in the strain map indicating intermediate hardness.

Several studies including two meta-analyses conclude that EUS elastography may help characterize focal pancreatic lesions as benign or malignant based on their imaged strain with an high accuracy[42-44]. In CP, the pathology is sometimes more diffusely distributed, and tissue hardness may not be limited to hypoechoic areas. Fibrosis and calcifications, if present, also increase tissue hardness in addition to local edema. In a transcutaneous shear wave study of acute pancreatitis Mateen *et al*[42] found that pancreatic tissue undergoes a cycle of increasing tissue hardness, resulting in low strain, in the acute

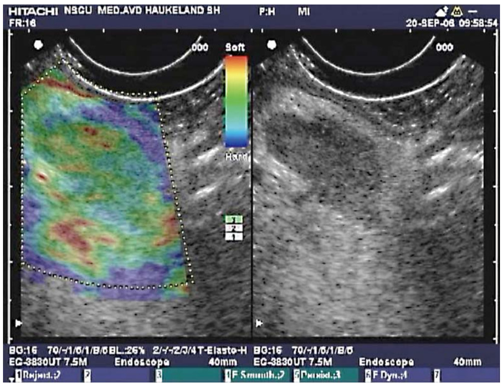

Figure 6 Endoscopic ultrasonography B-mode sonogram and elastogram of lymph node in chronic pancreatitis. The sonogram (right) shows a lymph node as a hypoechoic oval shape surrounded by more echogenic tissue in the liver hilum. This lymph node approximately 18 mm × 10 mm, appeared in the liver hilum of a patient with chronic pancreatitis. On the left, the lymph node is not harder than the surrounding tissue as the predominant color hue is green. This finding is frequent in reactive lymph nodes, and may be a sign of benign etiology.

phase of inflammation which subsequently turned softer as the inflammatory reaction resolved. In a study on focal pancreatic lesions we found that hypoechoic lesions that turned out to be inflammatory in origin were significantly softer than the malignant lesions. However, these lesions were imaged at a median of 8 mo after the last inflammatory attack (range: 1.2-12 mo). It is our experience that inflammatory lesions in the pancreas may present with a wide range of tissue strain. If a high-strain area (indicating soft tissue) is found, corresponding to a hypoechoic area in a sonogram, the chance of finding malignancy is however low (abstract Havre, UEGW 2011).

INFLAMMATORY DISEASES OF THE PANCREAS

In the following morphological and sonographic characteristics of pancreatic diseases with emphasis on CP will be outlined. Pancreatic neoplasms are also shortly described as an important differential diagnosis.

Acute pancreatitis

Acute pancreatitis is an acute inflammatory process that may include interstitial edema, necrosis, hemorrhage of pancreatic tissue and fluid collections, depending on the severity of the inflammation. The changes can be focal enlargement or diffuse, depending on its distribution and sometimes difficult to differentiate, especially when acute pancreatitis occurs in a patient with CP. Furthermore, focal pancreatitis often occurs in the pancreatic head, as a hypoechoic mass and not easily distinguishable from a tumor or changes seen in CP. The introduction of CEUS and its ability to differentiate between avascular necrotic tissue, cysts, abscesses and normal or hypervascular inflamed tissue equates US with CT[45]. CEUS has bedside availability, better cost effectiveness and can also be used in those patients where CT contrast agents are contraindicated. However, visualization of the whole gland can be a problem in very obese patients.

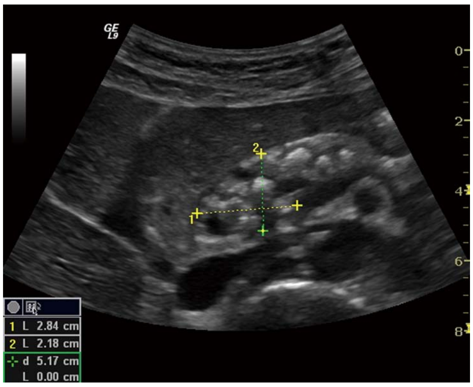

Figure 7 Advanced chronic pancreatitis. Classical signs in advanced chronic pancreatitis: main pancreatic duct dilatation in an atrophic organ with sharp, irregular contours, calcifications and small cysts. The pancreatic head is outlined.

Chronic pancreatitis

Classical diagnostic findings of CP in transabdominal ultrasonography reflects the spectrum of pancreatic disorders in CP. Ranging from progressive and irreversible morphological and functional derangement, inflammatory episodes and obstruction of structures adjacent to the pancreas[46].

Late stage, severe CP is normally easily recognizable due to characteristic morphological changes. The presences of pancreatic or intraductal calcifications presented as hyperechoic foci are pathognomonic[47]. Caliber abnormalities such as a dilated and irregular pancreatic duct is seen with a sensitivity of approximately 70%[48]. The reported sensitivity is probably due to limited duct changes in early/mild and moderate CP, where the pancreatic duct is less than 3 mm in diameter[49]. Pseudocysts may cause benign duct obstruction (stricture) and dilatation upstream[50]. Solid or cystic lesions and malignant infiltration, especially if contiguous to the main duct, may also cause duct compression, with progressive development of obstructive CP upstream. A small atrophic gland with focal alterations and parenchymal heterogeneity is easily identified in advanced stages. Figures 7 (B-mode US) and 8 (CEUS image of the pancreas in the early arterial phase) shows typical morphological changes in advanced CP.

In moderate to severe CP, the pancreatic gland echo texture is inhomogeneous and rough due to coexistence of fibrotic hyperechoic and hypoechoic focal inflammation signs[49,51,52]. These findings are reported to be observed in approximately 70% of the cases, already in the late eighties[48,53,54]. Echogenicity of the pancreas is usually increased in CP due to fibrosis and fatty infiltration. This is not a specific parameter due to presence of adipose tissue in elderly and obese patients[10,53]. Increased echogenicity of duct wall can also be detected[50]. Figure 9 show an example of elastography in moderate CP.

Early stage CP is normally without or with minimal

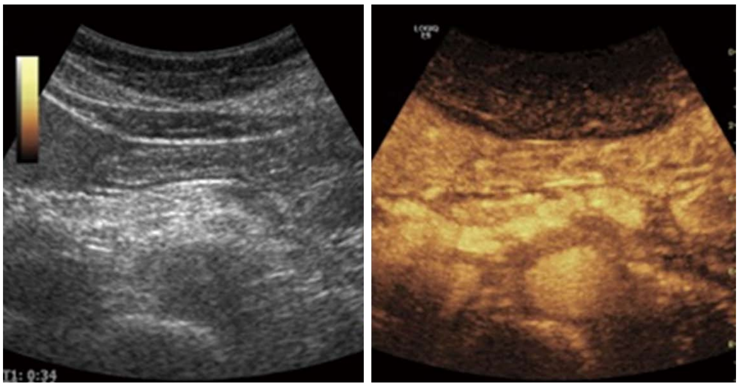

Figure 8 An example of contrast enhanced ultrasound in advanced chronic pancreatitis. The parenchymal enhancement is clearly irregular reflecting the parenchymal heterogeneity, calcifications and focal inflammation.

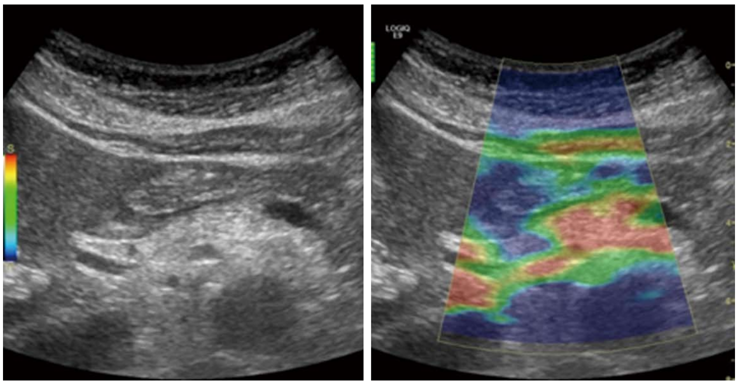

Figure 9 Elastography of the pancreas in moderate chronic pancreatitis. The colors show tissue hardness; The scale on the left defines the color code: Blue is hard, red is soft, yellow and green are intermediate. The elastogram shows predominantly soft (red) tissue with parts of green and yellow, indicating harder pancreatic tissue.

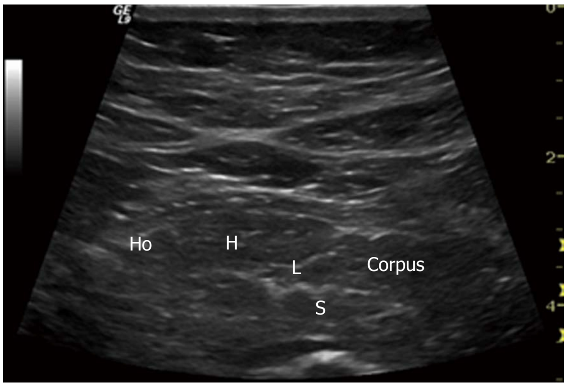

Figure 10 Early chronic pancreatitis. Typical signs in early chronic pancreatitis: lobularity (L), stranding (S), hyperechoic foci (H) and honeycombing (Ho). Pancreatic body (Corpus). This subtle changes are usually only seen in endoscopic ultrasonography.

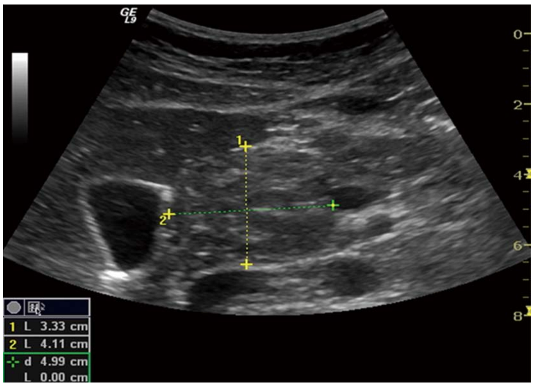

Figure 11 Autoimmune pancreatitis. Enlarged pancreas with a tumorous formation in the head. The inflammatory lesion is isoechoic to the rest of the pancreas but still clearly visible.

morphological changes, where imaging modality falls short in diagnosing CP. Late stages signs like calcifications or dilated and irregular main pancreatic duct are diagnostic for CP, but can also be absent in severe exocrine insufficiency, depending on the type of CP[55,56].

Cambridge classification of endoscopic retrograde pancreatography (ERP), US and CT imaging, grades the severity of CP, based on pancreatic structural changes and abnormalities of the main duct and side branches[52]. The sensitivity in diagnosing and grading the severity of CP with ultrasound alone, compared to the morphological gold standard ERP, rose to over 80% in the late eighties[53]. With the development of endosonographic ultrasound, subtle early changes in the pancreatic echostructure could be visualized, and a new gold standard, based on morphological criteria correlating with pathologic features emerged[57,58]. Current transabdominal high-end ultrasound scanners can visualize some of these criteria, although the Rosemont classification is only for EUS images. Figure 10 shows minimal morphological changes in a patient with early CP, obtained by transabdominal US. Forthcoming studies comparing transabdominal ultrasonography findings with EUS criteria seem to be a logical step forward.

Pancreatic functional tests are still the only option to diagnose an early pancreatitis without morphological changes but with physiological insufficiency. Today, the

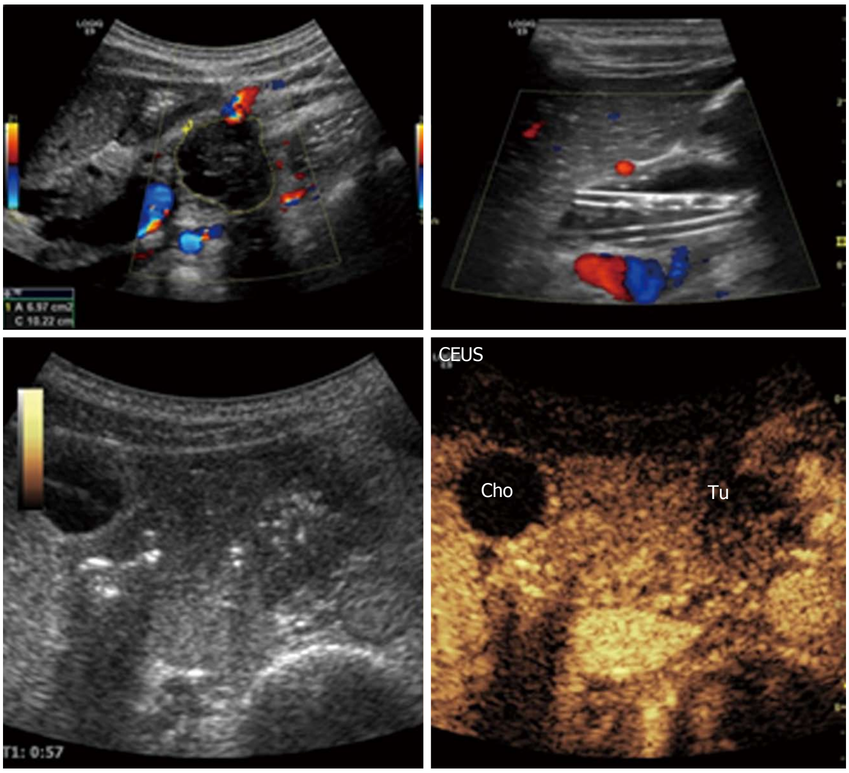

Figure 12 Malignant tumor of the pancreatic body: Ductal adenocarcinoma. The tumor is clearly visible on B-mode, left upper image. Contrast enhanced ultrasonography: The characteristics are hypoechogenicity and diffuse contours. The plastic biliary stents are clearly noticeable on B-mode, right upper image. Cho: Biliary duct (ductus choledochus); Tu: Tumor; CEUS: Contrast-enhanced ultrasonography.

short endoscopic secretin-based, pancreas function test is reasonably validated and the best available test[59,60]. This test can be combined with bedside US, to detect any morphological changes of the pancreas. Using this combined method we observed that the MPD wall had an increased echogenicity, *i.e.*, hyperechoic duct wall and changes in the diameter, short after secretin was given. Data not yet published.

Autoimmune pancreatitis

Autoimmune pancreatitis is a benign form with autoimmune pathogenesis and a remarkable response to steroid therapy. From an imaging point of view, there are three (focal, diffuse, and combined) types of autoimmune pancreatitis where, especially the focal type can be misinterpreted as cancer[61,62]. Figure 11 shows an enlarged pancreas with a round tumorous formation, without cysts and calcification. On CEUS the affected tissue is hyperemic and shows an increased contrast-enhancement, whereas pancreatic cancer lesions are typically hypervascularized[63]. The patient avoided excessive abdominal surgery and was successfully treated with corticosteroids.

Pseudocyst

Pseudocysts can arise as a complication of acute pancreatitis and also often occur in CP. These cysts are well-defined, fibrous-walled anechoic structures without an epithelial lining. Pseudocysts are normally easily differentiated from pancreatic cystic tumors, where especially the premalignant and malignant forms should be identified. CEUS can classify cystic tumor's or solid nodules through evaluation of the vascularization pattern or absence of vessels in pseudocysts[36].

Pancreatic solid neoplasm

Differentiation between mass-forming lesions in CP and adenocarcinomas of the pancreas are a daily challenge. The ultrasonographic findings are similar with homogeneous or inhomogeneous poorly defined hypoechoic mass in both cases[64]. After the initial detection by ultrasound, the subsequent use of CEUS, with its possibility to visualize the vascular pattern will improve the diagnostic accuracy of the lesion type. A ductal adenocarcinoma is typically hypoechoic in arterial phase without clear-cut margins, with optional central necrotic parts. On the contrary, CP has usually more vascularity, with the same enhancement patter as the surrounding pancreatic parenchyma[31,65,66]. Figure 12 shows a large hypervascularized mass with marked dilatation of the main bile duct. However, in advanced CP, due to fibrosis, inhomogenous hypervascularization can be present, making the differential diagnosis very difficult[35,67]. Differences in vascularity seen on CEUS, often correlate well with histology. Taking in account the higher temporal and spatial resolution of ultrasound into account makes CEUS equal or even superior to CT[32,34,38]. The relationship with the surrounding arterial and venous vessels, the presence of thrombosis of the portal or splenic vein are used for local staging and assessment of tumor resectability. The presence of liver metastases can also be evaluated during the late phase of the CEUS scanning procedure.

CONCLUSION

Today's modern high end ultrasound scanners are still the first line modality in abdominal and pancreatic imaging. Ultrasonography is highly available, relative cheap and can be repeated on a daily basis, if needed. Ultrasound technology is developing towards smaller and really mobile scanners, implying that ultrasonography can be present in the pockets of the doctors supporting the clinical examination. With the rapid technical development, different modalities and methods, which demands computer processed images can be combined. This means highly improved image quality and diagnostic accuracy. Under good scanning conditions, ultrasonography has higher spatial resolution than CT or MRI. Furthermore, CEUS with its ability to show macro- and micro vascularity is also used as a second line modality to clarify small lesions, previously detected on CT or MRI.

Regarding transabdominal ultrasound of the pancreas, we predict that the resolution and thereby the ability to detect small morphological changes will be approaching the performance of EUS. Elastography in pancreatic imaging is now well known in EUS and studies' regarding the usefulness of this method in transabdominal ultrasonography is warranted. The ability of CEUS to visualize and quantify perfusion and thereby to characterize a given lesion or to differentiate between vascular (solid) and avascular (liquid/necrotic) components of the lesion is one of the most important progresses in pancreatic imaging today. Furthermore, the development of three-dimensional technology offers new clinical possibilities for real-time 3D volume images of the pancreas in the future.

REFERENCES

1 **Gilja OH**, Hatlebakk JG, Odegaard S, Berstad A, Viola I, Giertsen C, Hausken T, Gregersen H. Advanced imaging and visualization in gastrointestinal disorders. *World J Gastroenterol* 2007; **13**: 1408-1421 [PMID: 17457973]

2 **Rösch T**, Schusdziarra V, Born P, Bautz W, Baumgartner M, Ulm K, Lorenz R, Allescher HD, Gerhardt P, Siewert JR, Classen M. Modern imaging methods versus clinical assessment in the evaluation of hospital in-patients with suspected pancreatic disease. *Am J Gastroenterol* 2000; **95**: 2261-2270 [PMID: 11007227]

3 **Swobodnik W**, Meyer W, Brecht-Kraus D, Wechsler JG, Geiger S, Malfertheiner P, Junge U, Ditschuneit H. Ultrasound, computed tomography and endoscopic retrograde cholangiopancreatography in the morphologic diagnosis of pancreatic disease. *Klin Wochenschr* 1983; **61**: 291-296 [PMID: 6865263 DOI: 10.1007/BF01497778]

4 **Rettenmaier G**. [Methods and results of ultrasonography in the diagnosis of pancreatic diseases]. *Leber Magen Darm* 1972; **2**: 88-90 [PMID: 5074453]

5 **Russell JG**, Vallon AG, Braganza JM, Howat HT. Ultrasonic scanning in pancreatic disease. *Gut* 1978; **19**: 1027-1033 [PMID: 730069 DOI: 10.1136/gut.19.11.1027]

6 **Reuss J**, Rettenmaier G. [Transabdominal ultrasonography in pancreatic diseases]. *Schweiz Med Wochenschr* 1993; **123**: 1049-1058 [PMID: 8511536]

7 **Fontana G**, Bolondi L, Conti M, Plicchi G, Gullo L, Caletti GC, Labo G. An evaluation of echography in the diagnosis of pancreatic disease. *Gut* 1976; **17**: 228-234 [PMID: 1269992 DOI: 10.1136/gut.17.3.228]

8 **Bamber J**, Cosgrove D, Dietrich CF, Fromageau J, Bojunga J, Calliada F, Cantisani V, Correas JM, D'Onofrio M, Drakonaki EE, Fink M, Friedrich-Rust M, Gilja OH, Havre RF, Jenssen C, Klauser AS, Ohlinger R, Saftoiu A, Schaefer F, Sporea I, Piscaglia F. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology. *Ultraschall Med* 2013; **34**: 169-184 [PMID: 23558397 DOI: 10.1055/s-0033-1335205]

9 **Cosgrove D**, Piscaglia F, Bamber J, Bojunga J, Correas JM, Gilja OH, Klauser AS, Sporea I, Calliada F, Cantisani V, D'Onofrio M, Drakonaki EE, Fink M, Friedrich-Rust M, Fromageau J, Havre RF, Jenssen C, Ohlinger R, Săftoiu A, Schaefer F, Dietrich CF. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: Clinical applications. *Ultraschall Med* 2013; **34**: 238-253 [PMID: 23605169 DOI: 10.1055/s-0033-1335375]

10 **Shmulewitz A**, Teefey SA, Robinson BS. Factors affecting image quality and diagnostic efficacy in abdominal sonography: a prospective study of 140 patients. *J Clin Ultrasound* 1993; **21**: 623-630 [PMID: 8227392 DOI: 10.1002/jcu.1870210909]

11 **Fiegler W**. [Effect of various parameters on the resolution potential behind obstructing fatty tissue in sonography]. *Rofo* 1983; **139**: 85-90 [PMID: 6409738 DOI: 10.1055/s-2008-1055846]

12 **Fiegler W**, Felix R, Langer M, Schultz E. Fat as a factor affecting resolution in diagnostic ultrasound: possibilities for improving picture quality. *Eur J Radiol* 1985; **5**: 304-309 [PMID: 3910434]

13 **Dietrich CF**. Ultraschall-kurs. 5 ed. Cologne: Deutscher Ärzteverlag, 2006

14 **Erchinger F**, Dimcevski G, Engjom T, Gilja OH. Transabdominal ultrasonography of the pancreas: basic and new aspects. *Imaging in Med* 2011; **3**: 411-422 [DOI: 10.2217/iim.11.36]

15 **Tjora E**, Wathle G, Erchinger F, Engjom T, Molven A, Aksnes L, Haldorsen IS, Dimcevski G, Raeder H, Njølstad PR. Exocrine pancreatic function in hepatocyte nuclear factor 1β-maturity-onset diabetes of the young (HNF1B-MODY) is only moderately reduced: compensatory hypersecretion from a hypoplastic pancreas. *Diabet Med* 2013; **30**: 946-955 [PMID: 23600988 DOI: 10.1111/dme.12190]

16 **Holalkere NS**, Soto J. Imaging of miscellaneous pancreatic pathology (trauma, transplant, infections, and deposition). *Radiol Clin North Am* 2012; **50**: 515-528 [PMID: 22560695 DOI: 10.1016/j.rcl.2012.03.011]

17 **Feigelson J**, Pécau Y, Poquet M, Terdjman P, Carrère J, Chazalette JP, Ferec C. Imaging changes in the pancreas in cystic fibrosis: a retrospective evaluation of 55 cases seen over a period of 9 years. *J Pediatr Gastroenterol Nutr* 2000; **30**: 145-151 [PMID: 10697132 DOI: 10.1097/00005176-200002000-00010]

18 **Hohl C**, Schmidt T, Honnef D, Günther RW, Haage P. Ultrasonography of the pancreas. 2. Harmonic imaging. *Abdom Imaging* 2007; **32**: 150-160 [PMID: 16850350 DOI: 10.1007/s00261-006-9017-z]

19 **Shapiro RS**, Wagreich J, Parsons RB, Stancato-Pasik A, Yeh HC, Lao R. Tissue harmonic imaging sonography: evaluation of image quality compared with conventional sonography. *AJR Am J Roentgenol* 1998; **171**: 1203-1206 [PMID: 9798848 DOI: 10.2214/ajr.171.5.9798848]

20 **Hohl C**, Schmidt T, Haage P, Honnef D, Blaum M, Staatz G, Guenther RW. Phase-inversion tissue harmonic imaging

compared with conventional B-mode ultrasound in the evaluation of pancreatic lesions. *Eur Radiol* 2004; **14**: 1109-1117 [PMID: 14714139 DOI: 10.1007/s00330-003-2191-2]

21 **Spârchez Z**. Tissue harmonic imaging: is it useful in hepatobiliary and pancreatic ultrasonography? *Rom J Gastroenterol* 2003; **12**: 239-246 [PMID: 14502326]

22 **Burns PN**. Harmonic imaging with ultrasound contrast agents. *Clin Radiol* 1996; **51** Suppl 1: 50-55 [PMID: 8605774]

23 **Köster J**, Schlosser T, Pohl C, Lentz C, Lohmaier S, Veltmann C, Kuntz-Hehner S, Omran H, Lüderitz B, Becher H, Tiemann K. Blood flow assessment by ultrasound-induced destruction of echocontrast agents using harmonic power Doppler imaging: which parameters determine contrast replenishment curves? *Echocardiography* 2001; **18**: 1-8 [PMID: 11182774 DOI: 10.1046/j.1540-8175.2001.00001.x]

24 **Bushberg JT**. Ultrasound. In: Bushberg JT, Seibert JA, Leidholdt EM, Boone JM, editors. The essential physics of medical imaging. 2 ed. Philadeplphia: Lippincott Williams & Wilkins, 2002: 543

25 **Gandolfi L**, Torresan F, Solmi L, Puccetti A. The role of ultrasound in biliary and pancreatic diseases. *Eur J Ultrasound* 2003; **16**: 141-159 [PMID: 12573783]

26 **Bertolotto M**, D'Onofrio M, Martone E, Malagò R, Pozzi Mucelli R. Ultrasonography of the pancreas. 3. Doppler imaging. *Abdom Imaging* 2007; **32**: 161-170 [PMID: 16865314]

27 **Forsberg F**. Ultrasonic biomedical technology; marketing versus clinical reality. *Ultrasonics* 2004; **42**: 17-27 [PMID: 15047256]

28 **Claudon M**, Cosgrove D, Albrecht T, Bolondi L, Bosio M, Calliada F, Correas JM, Darge K, Dietrich C, D'Onofrio M, Evans DH, Filice C, Greiner L, Jäger K, Jong Nd, Leen E, Lencioni R, Lindsell D, Martegani A, Meairs S, Nolsøe C, Piscaglia F, Ricci P, Seidel G, Skjoldbye B, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) - update 2008. *Ultraschall Med* 2008; **29**: 28-44 [PMID: 18270887 DOI: 10.1055/s-2007-963785]

29 **Claudon M**, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsøe CP, Piscaglia F, Wilson SR, Barr RG, Chammas MC, Chaubal NG, Chen MH, Clevert DA, Correas JM, Ding H, Forsberg F, Fowlkes JB, Gibson RN, Goldberg BB, Lassau N, Leen EL, Mattrey RF, Moriyasu F, Solbiati L, Weskott HP, Xu HX. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver--update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultraschall Med* 2013; **34**: 11-29 [PMID: 23129518 DOI: 10.1055/s-0032-1325499]

30 **Piscaglia F**, Nolsøe C, Dietrich CF, Cosgrove DO, Gilja OH, Bachmann Nielsen M, Albrecht T, Barozzi L, Bertolotto M, Catalano O, Claudon M, Clevert DA, Correas JM, D'Onofrio M, Drudi FM, Eyding J, Giovannini M, Hocke M, Ignee A, Jung EM, Klauser AS, Lassau N, Leen E, Mathis G, Saftoiu A, Seidel G, Sidhu PS, ter Haar G, Timmerman D, Weskott HP. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. *Ultraschall Med* 2012; **33**: 33-59 [PMID: 21874631 DOI: 10.1055/s-0031-1281676]

31 **Postema M**, Gilja OH. Contrast-enhanced and targeted ultrasound. *World J Gastroenterol* 2011; **17**: 28-41 [PMID: 21218081 DOI: 10.3748/wjg.v17.i1.28]

32 **D'Onofrio M**, Malagò R, Zamboni G, Vasori S, Falconi M, Capelli P, Mansueto G. Contrast-enhanced ultrasonography better identifies pancreatic tumor vascularization than helical CT. *Pancreatology* 2005; **5**: 398-402 [PMID: 15985763 DOI: 10.1159/000086540]

33 **Beyer-Enke SA**, Hocke M, Ignee A, Braden B, Dietrich CF. Contrast enhanced transabdominal ultrasound in the characterisation of pancreatic lesions with cystic appearance. *JOP* 2010; **11**: 427-433 [PMID: 20818109]

34 **Kersting S**, Konopke R, Kersting F, Volk A, Distler M, Bergert H, Saeger HD, Grützmann R, Bunk A. Quantitative perfusion analysis of transabdominal contrast-enhanced ultrasonography of pancreatic masses and carcinomas. *Gastroenterology* 2009; **137**: 1903-1911 [PMID: 19715694 DOI: 10.1053/j.gastro.2009.08.049]

35 **D'Onofrio M**, Zamboni G, Tognolini A, Malago R, Faccioli N, Frulloni L, Pozzi Mucelli R. Mass-forming pancreatitis: value of contrast-enhanced ultrasonography. *World J Gastroenterol* 2006; **12**: 4181-4184 [PMID: 16830370]

36 **D'Onofrio M**, Martone E, Malagò R, Faccioli N, Zamboni G, Comai A, Cugini C, Gubello T, Pozzi Mucelli R. Contrast-enhanced ultrasonography of the pancreas. *JOP* 2007; **8**: 71-76 [PMID: 17228138]

37 **D'Onofrio M**, Mansueto G, Falconi M, Procacci C. Neuroendocrine pancreatic tumor: value of contrast enhanced ultrasonography. *Abdom Imaging* 2004; **29**: 246-258 [PMID: 15290954 DOI: 10.1007/s00261-003-0097-8]

38 **Kersting S**, Roth J, Bunk A. Transabdominal contrast-enhanced ultrasonography of pancreatic cancer. *Pancreatology* 2011; **11** Suppl 2: 20-27 [PMID: 21464583 DOI: 10.1159/000323480]

39 **Kotopoulis S**, Dimcevski G, Gilja OH, Hoem D, Postema M. Treatment of human pancreatic cancer using combined ultrasound, microbubbles, and gemcitabine: a clinical case study. *Med Phys* 2013; **40**: 072902 [PMID: 23822453 DOI: 10.1118/1.4808149]

40 **Albashir S**, Bronner MP, Parsi MA, Walsh RM, Stevens T. Endoscopic ultrasound, secretin endoscopic pancreatic function test, and histology: correlation in chronic pancreatitis. *Am J Gastroenterol* 2010; **105**: 2498-2503 [PMID: 20606675 DOI: 10.1038/ajg.2010.274]

41 **Catalano MF**, Sahai A, Levy M, Romagnuolo J, Wiersema M, Brugge W, Freeman M, Yamao K, Canto M, Hernandez LV. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. *Gastrointest Endosc* 2009; **69**: 1251-1261 [PMID: 19243769 DOI: 10.1016/j.gie.2008.07.043]

42 **Mateen MA**, Muheet KA, Mohan RJ, Rao PN, Majaz HM, Rao GV, Reddy DN. Evaluation of ultrasound based acoustic radiation force impulse (ARFI) and eSie touch sonoelastography for diagnosis of inflammatory pancreatic diseases. *JOP* 2012; **13**: 36-44 [PMID: 22233945]

43 **Săftoiu A**, Vilmann P, Gorunescu F, Janssen J, Hocke M, Larsen M, Iglesias-Garcia J, Arcidiacono P, Will U, Giovannini M, Dietrich CF, Havre R, Gheorghe C, McKay C, Gheonea DI, Ciurea T. Efficacy of an artificial neural network-based approach to endoscopic ultrasound elastography in diagnosis of focal pancreatic masses. *Clin Gastroenterol Hepatol* 2012; **10**: 84-90.e1 [PMID: 21963957 DOI: 10.1016/j.cgh.2011.09.014]

44 **Săftoiu A**, Vilmann P, Gorunescu F, Janssen J, Hocke M, Larsen M, Iglesias-Garcia J, Arcidiacono P, Will U, Giovannini M, Dietrich C, Havre R, Gheorghe C, McKay C, Gheonea DI, Ciurea T. Accuracy of endoscopic ultrasound elastography used for differential diagnosis of focal pancreatic masses: a multicenter study. *Endoscopy* 2011; **43**: 596-603 [PMID: 21437851 DOI: 10.1055/s-0030-1256314]

45 **Rickes S**, Mönkemüller K, Malfertheiner P. Acute severe pancreatitis: contrast-enhanced sonography. *Abdom Imaging* 2007; **32**: 362-364 [PMID: 17514345 DOI: 10.1007/s00261-007-9250-0]

46 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244 DOI: 10.1053/gast.2001.22586]

47 **Homma T**, Harada H, Koizumi M. Diagnostic criteria for chronic pancreatitis by the Japan Pancreas Society. *Pancreas* 1997; **15**: 14-15 [PMID: 9211487]

48 **Bolondi L**, Priori P, Gullo L, Santi V, Li Bassi S, Barbara L, Labó G. Relationship between morphological changes detected by ultrasonography and pancreatic exocrine func-

tion in chronic pancreatitis. *Pancreas* 1987; **2**: 222-229 [PMID: 3306660]
49 **Remer EM**, Baker ME. Imaging of chronic pancreatitis. *Radiol Clin North Am* 2002; **40**: 1229-142, v [PMID: 12479708 DOI: 10.1016/S0033-8389(02)00044-1]
50 **Axon AT**, Classen M, Cotton PB, Cremer M, Freeny PC, Lees WR. Pancreatography in chronic pancreatitis: international definitions. *Gut* 1984; **25**: 1107-1112 [PMID: 6479687 DOI: 10.1136/gut.25.10.1107]
51 **Siddiqi AJ**, Miller F. Chronic pancreatitis: ultrasound, computed tomography, and magnetic resonance imaging features. *Semin Ultrasound CT MR* 2007; **28**: 384-394 [PMID: 17970554 DOI: 10.1053/j.sult.2007.06.003]
52 **Sarner M**, Cotton PB. Classification of pancreatitis. *Gut* 1984; **25**: 756-759 [PMID: 6735257 DOI: 10.1136/gut.25.7.756]
53 **Jones SN**, Lees WR, Frost RA. Diagnosis and grading of chronic pancreatitis by morphological criteria derived by ultrasound and pancreatography. *Clin Radiol* 1988; **39**: 43-48 [PMID: 3276430 DOI: 10.1016/S0009-9260(88)80339-8]
54 **Alpern MB**, Sandler MA, Kellman GM, Madrazo BL. Chronic pancreatitis: ultrasonic features. *Radiology* 1985; **155**: 215-219 [PMID: 3883420]
55 **Ammann RW**, Muench R, Otto R, Buehler H, Freiburghaus AU, Siegenthaler W. Evolution and regression of pancreatic calcification in chronic pancreatitis. A prospective long-term study of 107 patients. *Gastroenterology* 1988; **95**: 1018-1028 [PMID: 3410215]
56 **Lankisch PG**, Otto J, Erkelenz I, Lembcke B. Pancreatic calcifications: no indicator of severe exocrine pancreatic insufficiency. *Gastroenterology* 1986; **90**: 617-621 [PMID: 3943692]
57 **Sahai AV**. EUS and chronic pancreatitis. *Gastrointest Endosc* 2002; **56**: S76-S81 [PMID: 12297754 DOI: 10.1016/S0016-5107(02)70091-6]
58 **Seicean A**. Endoscopic ultrasound in chronic pancreatitis: where are we now? *World J Gastroenterol* 2010; **16**: 4253-4263 [PMID: 20818808 DOI: 10.3748/wjg.v16.i34.4253]
59 **Stevens T**, Dumot JA, Zuccaro G, Vargo JJ, Parsi MA, Lopez R, Kirchner HL, Purich E, Conwell DL. Evaluation of duct-cell and acinar-cell function and endosonographic abnormalities in patients with suspected chronic pancreatitis. *Clin Gastroenterol Hepatol* 2009; **7**: 114-119 [PMID: 18955165 DOI: 10.1016/j.cgh.2008.09.002]
60 **Erchinger F**, Engjom T, Tjora E, Hoem D, Hausken T, Gilja OH, Dimcevski G. Quantification of pancreatic function using a clinically feasible short endoscopic secretin test. *Pancreas* 2013; **42**: 1101-1106 [PMID: 23921960 DOI: 10.1097/MPA.0b013e3182847a86]
61 **Rehnitz C**, Klauss M, Singer R, Ehehalt R, Werner J, Büchler MW, Kauczor HU, Grenacher L. Morphologic patterns of autoimmune pancreatitis in CT and MRI. *Pancreatology* 2011; **11**: 240-251 [PMID: 21625195 DOI: 10.1159/000327708]
62 **Law R**, Bronner M, Vogt D, Stevens T. Autoimmune pancreatitis: a mimic of pancreatic cancer. *Cleve Clin J Med* 2009; **76**: 607-615 [PMID: 19797461 DOI: 10.3949/ccjm.76a.09039]
63 **Hocke M**, Ignee A, Dietrich CF. Contrast-enhanced endoscopic ultrasound in the diagnosis of autoimmune pancreatitis. *Endoscopy* 2011; **43**: 163-165 [PMID: 21165827]
64 **Yassa NA**, Yang J, Stein S, Johnson M, Ralls P. Gray-scale and color flow sonography of pancreatic ductal adenocarcinoma. *J Clin Ultrasound* 1997; **25**: 473-480 [PMID: 9350565]
65 **Dietrich CF**, Braden B, Hocke M, Ott M, Ignee A. Improved characterisation of solitary solid pancreatic tumours using contrast enhanced transabdominal ultrasound. *J Cancer Res Clin Oncol* 2008; **134**: 635-643 [PMID: 17952469 DOI: 10.1007/s00432-007-0326-6]
66 **Faccioli N**, Crippa S, Bassi C, D'Onofrio M. Contrast-enhanced ultrasonography of the pancreas. *Pancreatology* 2009; **9**: 560-566 [PMID: 19657211 DOI: 10.1159/000225960]
67 **Takeda K**, Goto H, Hirooka Y, Itoh A, Hashimoto S, Niwa K, Hayakawa T. Contrast-enhanced transabdominal ultrasonography in the diagnosis of pancreatic mass lesions. *Acta Radiol* 2003; **44**: 103-106 [PMID: 12631008]

P- Reviewers: Lee SW, Neri V **S- Editor**: Wen LL **L- Editor**: A
E- Editor: Zhang DN

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7258

World J Gastroenterol 2013 November 14; 19(42): 7258-7266
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Diagnosis and treatment of pancreatic exocrine insufficiency

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Received: June 20, 2013 **Revised:** August 22, 2013
Accepted: September 16, 2013
Published online: November 14, 2013

Abstract

Pancreatic exocrine insufficiency is an important cause of maldigestion and a major complication in chronic pancreatitis. Normal digestion requires adequate stimulation of pancreatic secretion, sufficient production of digestive enzymes by pancreatic acinar cells, a pancreatic duct system without significant outflow obstruction and adequate mixing of the pancreatic juice with ingested food. Failure in any of these steps may result in pancreatic exocrine insufficiency, which leads to steatorrhea, weight loss and malnutrition-related complications, such as osteoporosis. Methods evaluating digestion, such as fecal fat quantification and the ^{13}C-mixed triglycerides test, are the most accurate tests for pancreatic exocrine insufficiency, but the probability of the diagnosis can also be estimated based on symptoms, signs of malnutrition in blood tests, fecal elastase 1 levels and signs of morphologically severe chronic pancreatitis on imaging. Treatment for pancreatic exocrine insufficiency includes support to stop smoking and alcohol consumption, dietary consultation, enzyme replacement therapy and a structured follow-up of nutritional status and the effect of treatment. Pancreatic enzyme replacement therapy is administered in the form of enteric-coated minimicrospheres during meals. The dose should be in proportion to the fat content of the meal, usually 40-50000 lipase units per main meal, and half the dose is required for a snack. In cases that do not respond to initial treatment, the doses can be doubled, and proton inhibitors can be added to the treatment. This review focuses on current concepts of the diagnosis and treatment of pancreatic exocrine insufficiency.

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Key words: Chronic pancreatitis; Pancreatic exocrine insufficiency; Pancreatic enzyme replacement therapy

Core tip: This is a review on the diagnosis and treatment of pancreatic exocrine insufficiency. The review includes a discussion of the definition of pancreatic exocrine insufficiency, a pragmatic approach to its diagnosis and current concepts of indications for treatment with pancreatic enzyme replacement therapy, including measures to optimize the effect.

Lindkvist B. Diagnosis and treatment of pancreatic exocrine insufficiency. *World J Gastroenterol* 2013; 19(42): 7258-7266 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7258.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7258

INTRODUCTION

Pancreatic exocrine insufficiency (PEI) can be defined as a reduction in pancreatic enzyme activity in the intestinal lumen to a level that is below the threshold required to maintain normal digestion. This concept is crucial for the understanding of PEI and has several important consequences for the diagnosis and treatment of this

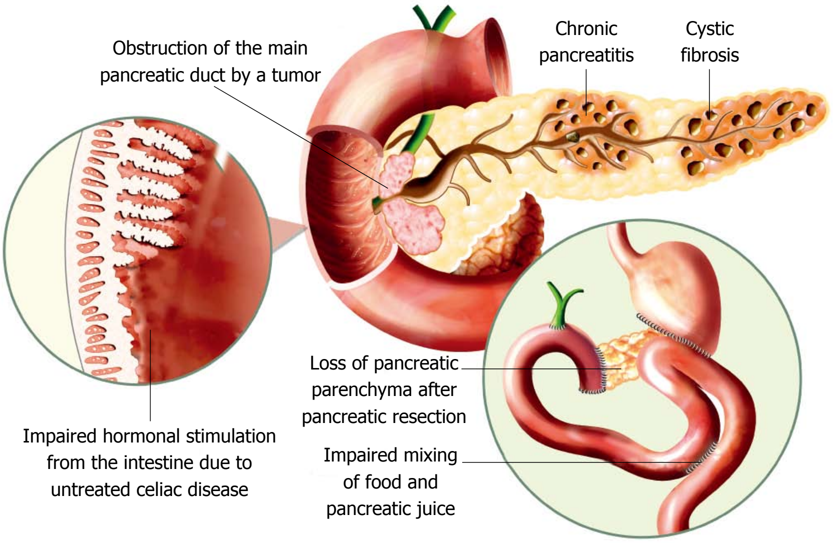
Obstruction of the main pancreatic duct by a tumor
Chronic pancreatitis
Cystic fibrosis
Impaired hormonal stimulation from the intestine due to untreated celiac disease
Loss of pancreatic parenchyma after pancreatic resection
Impaired mixing of food and pancreatic juice

Figure 1 Different causes of pancreatic exocrine insufficiency.

condition. First, pancreatic exocrine secretion can be significantly reduced without PEI being present. In a landmark paper four decades ago, DiMagno *et al*[1] demonstrated that steatorrhea does not occur until pancreatic lipase output is reduced to 5%-10% of normal output. Hence, the demonstration of moderately reduced bicarbonate or enzyme output in sensitive tests of pancreatic secretion, such as the secretin/cholecystokinin-stimulation test, is a reliable indicator of chronic pancreatitis (CP) but does not necessarily indicate PEI. Second, any pathology, including extrapancreatic conditions, that interrupt the chain of events required for the normal digestion of ingested food by pancreatic digestive enzymes may cause PEI. Thus, “pancreatic exocrine insufficiency” is a denomination that, from a semantic point of view, is too narrow for this condition; “pancreatic maldigestion” could be an alternative and probably more correct term. Diseases of the pancreatic parenchyma, such as CP, cystic fibrosis and status post necrotizing acute pancreatitis, are the most common causes of PEI. However, PEI may also be caused by obstruction of the pancreatic duct system due to a tumor or a stricture, by reduced stimulatory capacity in the intestine secondary to untreated celiac disease[2] or Crohn’s disease, by increased intraluminal inactivation of pancreatic enzymes in Zollinger-Ellison syndrome[3] or by impaired mixing of ingested food and the pancreatic juice after upper gastrointestinal surgery[4] (Figure 1).

The pancreatic juice plays a pivotal role in the digestion and absorption of nutrients[5]. Pancreatic enzyme secretion is stimulated during the cephalic[6] and gastric[7] phases to a certain degree, but the most important stimulation occurs during the intestinal phase, when chyme enters the duodenum. The presence of fatty acids, amino acids and gastric acid in the duodenum is the most potent stimulator of exocrine pancreatic secretion[8]. Vagal and neural reflexes stimulate pancreatic secretion during the cephalic and gastric phases[6,7]. During the intestinal phase, cells in the duodenal mucosa release CCK, which stimulates the secretion of pancreatic enzymes from acinar cells[9], and secretin, which elicits water and bicarbonate secretion from ductal cells[10,11].

The pancreatic juice consists of bicarbonate and water secreted by ductal cells and several enzymes, secreted by acinar cells, with the specific capacity to digest proteins, carbohydrates and fat. In situations with reduced exocrine pancreatic function, the ability to digest fat is the determining factor that causes the most important symptoms and clinical complications because lipase, the major lipolytic enzyme of the pancreatic juice, is the pancreatic digestive enzyme with the poorest stability in the gastrointestinal lumen. The destruction of lipase is even more rapid when the pH is below 4, which is often the situation in CP, in which the buffering of gastric acid is insufficient due to low bicarbonate excretion by the pancreas[12]. Furthermore, there is minimal extrapancreatic lipolytic enzyme production, as opposed to the extrapancreatic capacity to digest carbohydrates provided by salivary amylase and intestinal oligosaccharidoses or the proteolytic capacity provided by gastric pepsinogen.

PEI is one of the major complications in CP and should be considered in all CP patients. The prevalence of PEI in CP increases with disease duration, and approximately half of patients will have developed PEI by 12 years after disease onset[13]. There are no reliable estimates of the prevalence of PEI in the general population.

Patients with untreated PEI not only suffer from impaired quality of life due to steatorrhea, weight loss, abdominal discomfort and other PEI-related symptoms but are also highly likely to develop deficiencies of micronutrients and lipid-soluble vitamins[14]. These deficiencies in turn place patients at risk of malnutrition-related complications, such as osteoporosis[15,16]. Hence, an early and accurate diagnosis of PEI is of high clinical importance.

DIAGNOSIS OF PANCREATIC EXOCRINE INSUFFICIENCY

The gold standard for the diagnosis of PEI is three-day fecal fat quantification and determination of the coefficient of fat absorption[17]. A major drawback of fecal fat quantification is that the test is cumbersome and unpleasant for both the patient and laboratory personnel. The patient is required to keep a strict diet, with 100 g of fat per day for five days, and to collect the complete volume of feces for three days. Laboratory personnel need to handle large volumes of feces. Therefore, this test is very rarely performed in daily clinical practice and is only available at few specialized centers. Several alternative methods for the diagnosis of PEI have been proposed.

Diagnosis based on symptoms, blood tests and imaging

Symptoms in patients with PEI vary, depending on the degree and etiology of PEI. The classical clinical picture is a patient presenting with foul-smelling, loose stools, weight loss, muscle wasting; and flatulence. Advanced tests of pancreatic exocrine function can usually be avoided in patients with a well-established CP diagnosis based on morphological findings and a clear clinical picture of PEI. A trial of pancreatic enzyme replacement therapy (PERT) based only on the clinical picture is recommended by several national societies when the clinical presentation is strongly suggestive of PEI[18,19]. However, only relying on symptoms may lead to both the over- and under-diagnosis of PEI. Diarrhea and weight loss may be due to conditions other than PEI, and PEI can also be present in the absence of overt steatorrhea.

In addition to explaining and treating clinical symptoms, the second rationale for the early diagnosis of PEI is to prevent complications of malnutrition. It is reasonable to assume that such malnutrition-related complications will be preceded by deficiencies of macro- or micronutrients detectable by routine blood tests. Hence, from a theoretical point of view, serum nutritional markers could be used to support the diagnosis of PEI. Deficiencies of several nutrients in blood tests have been demonstrated in CP, including apolipoproteins[20,21], total cholesterol[22], magnesium[22,23], lipid-soluble vitamins[24-26], retinol-binding protein[26], calcium, zinc and selenium[25], but the majority of these studies have not taken the exocrine function status of patients into consideration. Studies investigating the association between nutritional markers and PEI in CP patients have demonstrated that deficiencies of lipid-soluble vitamins are associated with an increased probability of PEI[15,26], as opposed to B12 and folate levels, which are not associated with PEI[14,27]. The possibility of diagnosing PEI based on nutritional markers in the blood was recently studied in a cohort of 114 patients with CP, of whom 38 suffered from PEI[14]. Hemoglobin, albumin, prealbumin and retinol-binding protein levels below the lower limit of normal magnesium levels below 2.05 mg/dL; and HbA1C levels above the upper limit of normal were all significantly associated with PEI. No PEI patient in this study presented with normal values for all of these parameters. The central conclusion that can be drawn from this study is that a normal panel of serum nutritional markers can exclude PEI with a high negative predictive value.

The probability of PEI in CP can also be estimated based on pancreatic imaging findings in the absence of more advanced tests of pancreatic function[28]. Notably, ductal changes on endoscopic retrograde pancreatography, computerized tomography (CT)[29] and endoscopic ultrasound (EUS) have been associated with decreased exocrine pancreatic function. The diagnosis of CP by EUS is based on the demonstration of several different parenchymal (hyperechoic foci, hyperechoic strands, parenchymal lobularity and cysts) and ductal (pancreatic duct dilatation, irregular pancreatic duct contour, hyperechoic pancreatic duct margin, dilated side branches and intraductal calcifications) abnormalities defined in the Rosemont classification[30]. A recent study demonstrated a clear correlation between the number of EUS criteria met and the probability of PEI. Calcifications and main pancreatic duct dilatation were independently associated with PEI in a multivariate analysis, and the probability of PEI was > 80% if these features were present[28].

Fecal elastase 1

Pancreatic elastase 1 is an enzyme of the pancreatic juice that is highly stable during passage through the gastrointestinal tract[31]. The concentration of elastase 1 can be measured in feces using a simple enzyme-linked immunosorbent assay [fecal elastase 1 (FE-1)] on a spot fecal sample[32,33]. FE-1 levels have been demonstrated to correlate with more sensitive tests of pancreatic secretion, such as the secretin test[32,34]. Low FE-1 levels have also been demonstrated to correlate with morphological tests for CP, such as endoscopic retrograde pancreatography[35] and magnetic resonance cholangiopancreatography[36]. However, as opposed to the relatively large number of studies evaluating FE-1 assessment as a test for the diagnosis of CP, studies evaluating the role of FE-1 testing in the detection of PEI in CP are scarce. Recently, Benini *et al*[37] investigated FE-1 and fecal fat in patients with CP or pancreatic resection. Three important conclusions can be drawn from this study. First, an FE-1 concentration of < 15 μg/g feces detects PEI with high sensitivity and specificity in patients with CP without prior pancreatic surgery. Second, intermediate FE-1 values (15-200 μg/g feces) are more difficult to interpret and likely warrant testing with more sensitive methods. Third, FE-1 assessment is not a reliable test for PEI in patients post-pancreatic resection. The fecal fat concentration was consistently higher in relation to FE-1 levels in operated compared with non-operated cases[37]. This finding was not unexpected; inadequate mixing of food with the pancreatic juice and other factors not related to pancreatic secretory capacity are likely to contribute to the pathogenesis of PEI after pancreatic resections.

^{13}C-mixed triglycerides breath test

The ^{13}C-mixed triglycerides (^{13}C-MTG) breath test was introduced by Vantrappen *et al*[38]. The test directly mea-

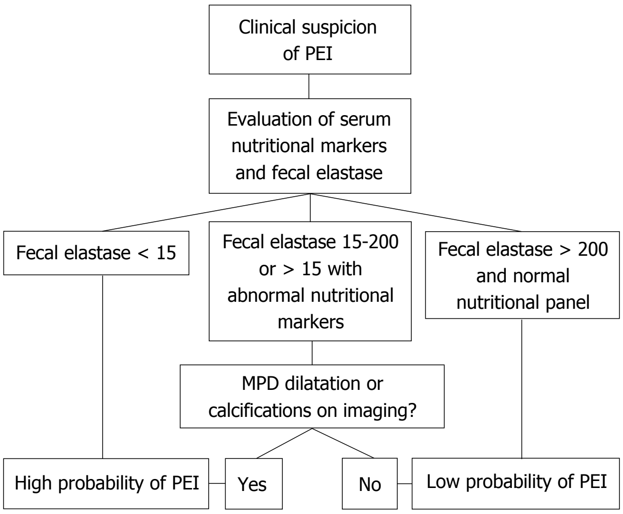
Clinical suspicion of PEI
Evaluation of serum nutritional markers and fecal elastase
Fecal elastase < 15
Fecal elastase 15-200 or > 15 with abnormal nutritional markers
Fecal elastase > 200 and normal nutritional panel
MPD dilatation or calcifications on imaging?
High probability of PEI
Yes
No
Low probability of PEI

Figure 2 Evaluation of the probability of pancreatic exocrine insufficiency in the absence of advanced tests for maldigestion, such as fecal fat quantification and the ^{13}C-mixed triglycerides breath test. PEI: Pancreatic exocrine insufficiency; MPD: Main pancreatic duct.

sures the clinically most relevant end-effect of exocrine pancreatic function: the degradation of triglycerides. This makes this test preferable to tests that measure exocrine pancreatic secretion, such as the secretin test and the FE-1 assay. For the ^{13}C-MTG test, the patient ingests a small amount of ^{13}C-marked triglycerides (2-octanoyl (1-^{13}C)-1,3 distearoyl glycerol), together with butter on a piece of toasted bread, after an overnight fast. In the presence of normal lipase activity, ^{13}C-triglycerides will be degraded in the intestinal lumen, and ^{13}C-marked fatty acids will then be absorbed. These fatty acids will in turn be metabolized in the liver, and $^{13}CO_2$ can finally be measured in exhaled air. Subjects with PEI have decreased lipase activity, which can be detected as a decreased recovery of $^{13}CO_2$ in exhaled air. Currently, there is no general agreement on the optimal design of the test and several different protocols have been proposed[39-43]. The protocol developed by Domínguez-Muñoz *et al*[44] has been adopted by several groups, including our institution, and this protocol is described in Table 1[44]. Values below 29% are considered as pathological, and the test detects fat maldigestion with a sensitivity of > 90%[40].

Test based on analysis of pancreatic juice after secretin/cerulein stimulation

Exocrine pancreatic function can be measured by so-called direct pancreatic function tests. In these tests, pancreatic secretion is stimulated by secretin and/or cerulein[45] or by the ingestion of a standard test meal[46]. After stimulation, samples of the pancreatic juice are aspirated from a tube that has been placed in the duodenum, and the concentrations of pancreatic digestive enzymes and bicarbonate are measured. A peak bicarbonate concentration in pancreatic secretion significantly below normal values (a cut-off value of 80 mEq has been advocated by most authorities) in the secretin test has long been considered as the most sensitive test for early CP. A drawback of the direct function tests is that they require the placement of a large-bore tube in the duodenum during the complete duration of the test, which is poorly tolerated by patients. It is also important to keep in mind that a mild reduction in pancreatic exocrine function, occasionally called "exocrine pancreatic dysfunction" or "mild pancreatic exocrine insufficiency", is not equivalent to clinically significant PEI. PEI, based on its definition, is a reduction in exocrine pancreatic function to a level that results in maldigestion. Since the introduction of highly sensitive pancreatic imaging methods, such as MRI, modern CT and endoscopic ultrasound, the need to rely on pancreatic function testing for the diagnosis of CP has diminished, and most centers have abandoned the classic secretin test.

Recently, an endoscopic direct pancreatic function test was developed, with a simplified protocol for pancreatic fluid collection through an endoscope. A good correlation between the endoscopic pancreatic function test and standard direct tests has been demonstrated[47,48]. Exocrine pancreatic secretion can also be evaluated based on the degree of duodenal filling on MRI after secretin stimulation, which has been demonstrated to correlate with a combination of the FE-1 assay, the ^{13}C-MTG breath test and fecal fat testing[49] and the endoscopic pancreatic function test[50]. Further studies are needed to evaluate the role of the endoscopic function test and secretin-enhanced MRI in the diagnosis of PEI. It should be noted that all tests of pancreatic secretion share the drawback of ignoring other factors that may alter the effect of pancreatic enzymes in the intestine. Thus, tests of pancreatic secretion are irrelevant in situations in which factors other than the secretory capacity of the pancreas may contribute to PEI.

Integrated use of methods to diagnose PEI

The optimal test for the diagnosis of PEI is a test that can detect the maldigestion of fat with high sensitivity and specificity. Fecal fat quantification and the ^{13}C-MTG breath test are recommended for the accurate diagnosis of PEI because these two tests best fit this description. However, due to the limited availability of these tests and the odious nature of fecal fat quantification, there is a need for a simplified way to estimate the probability of PEI based on generally available clinical parameters. In Figure 2, a proposal for a diagnostic algorithm to estimate the probability of PEI based on routine blood tests, FE-1 assessment and standard imaging is presented. The algorithm is designed to have a high negative predictive value for PEI and integrates knowledge from recent studies[14,28,37]. However, it should be stressed that this algorithm provides only an estimation of the probability of PEI and that the algorithm has not been scientifically validated.

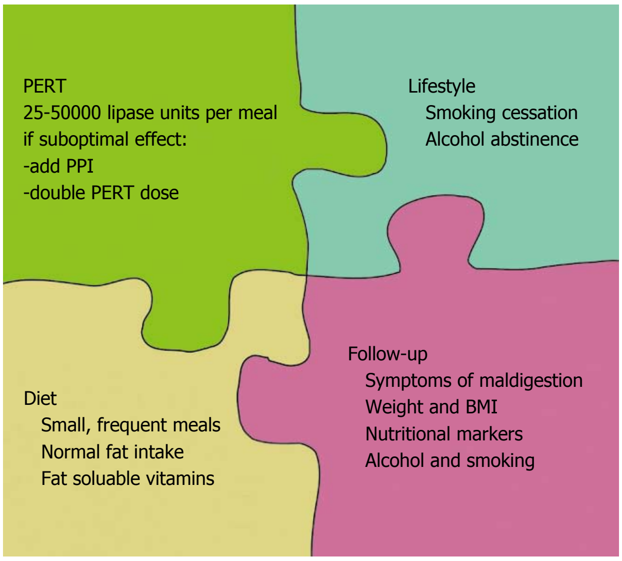

Figure 3 Fundamental aspects in the care of patients with pancreatic exocrine insufficiency. PERT: Pancreatic enzyme replacement therapy; PPI: Proton pump inhibitors.

TREATMENT OF PANCREATIC EXOCRINE INSUFFICIENCY

Cornerstones in the treatment of PEI are PERT, support to cease smoking and alcohol consumption, consultation with a dietitian and a systematic follow-up to assure optimal treatment effect (Figure 3). The goal of this treatment concept is to normalize digestion, alleviate PEI-related symptoms and prevent malnutrition-related morbidity and mortality and disease progression.

Diet, smoking and drinking

Historically, a low-fat diet has been recommended in PEI to reduce steatorrhea. This recommendation has been abandoned in modern dietary counseling in PEI due to the risk of aggravating PEI-related weight loss and deficiencies of lipid-soluble vitamins[18,51]. By optimization of the PERT dose and supportive treatment with PPI, most PEI patients will tolerate a normal-fat diet. Dietary consultation should include advice for sufficient caloric intake and normal fat content. Small, frequent meals are usually better tolerated than large, high-caloric meals. Deficiencies of fat-soluble vitamins are very common in PEI patients, and vitamin supplementation therapy should be given if necessary[15]. Support for alcohol abstinence should be offered to all patients with alcohol-related CP. In addition to the general health benefits of alcohol withdrawal, this withdrawal has also been demonstrated to slow the further deterioration of pancreatic exocrine function[52]. Smoking is a risk factor for pancreatic cancer, acute pancreatitis and CP[53], and is also associated with an increased probability of reduced pancreatic exocrine function based on the endoscopic pancreatic function test in cases with CP[54]. Continued smoking has been associated with earlier development of calcifications in patients with CP[55]. Smoking cessation should be encouraged in all patients with CP with or without PEI.

Pancreatic enzyme replacement therapy

Pancreatic digestive enzymes can be administered orally, together with meals, in patients with PEI to compensate for the lack of endogenous enzyme secretion. Modern pancreatic enzyme preparations are extracts from the porcine pancreas (pancrelipase) that are administered as enteric-coated minimicrospheres. Several different preparations are commercially available, with minor differences in particle size and pH-related release kinetics[56]. PEI with steatorrhea and/or weight loss is an undisputed indication for PERT. Several studies have demonstrated an improved coefficient of fat absorption[57-60], decreased maldigestion-related symptoms[57,59-61] and even improved quality of life[61,62]. The need for PERT in PEI without symptoms is a matter of debate, and randomized clinical trials on this issue are lacking. Nevertheless, certain collateral evidence supports PERT, even in the absence of overt steatorrhea and weight loss: (1) A longitudinal study in patients with CP demonstrated that patients with no clinical symptoms of steatorrhea but an abnormal steatocrit who did not receive PERT lost more weight than not only CP patients with a normal steatocrit but also patients with an abnormal steatocrit and symptoms of steatorrhea who were treated with PERT[63]; (2) Laboratory signs of malnutrition have been demonstrated in a large proportion of patients with CP and asymptomatic steatorrhea who were not under treatment with PERT[64]. The clinical relevance of such deficiencies has not been specifically investigated in CP and PEI, but an increased risk of complications that are generally associated with malnutrition can be assumed[65]; and (3) Based on observational studies in CP, it is well known that malnutrition-related diseases and findings, such as osteoporosis-related fractures[16], decreased bone mineral density[15,66,67] and deficiencies of fat-soluble vitamins[15], are common in CP.

As evidence for nutritional deficiencies in patients with PEI with and without symptoms has increased in recent years, PERT is now increasingly regarded as a treatment for maldigestion rather than a way to suppress diarrhea in patients with CP[65]. The goal of PERT is stated to be the elimination of maldigestion in the Australasian Pancreatic Club recommendations[18]. Meanwhile, the Spanish Pancreatic Club regards any clinical or nutritional deficiency in a CP patient as an indication for PERT[68], and the Italian Association for the Study of the Pancreas states in its guidelines that PEI is an indication for PERT[19].

The safety and efficacy of PERT for the treatment of PEI in CP has been investigated in four randomized, double-blinded, placebo-controlled clinical trials including up to 72 patients over study periods of 1-2 wk[57-60]. Significant improvements in the coefficient of fat absorption[57-60], the coefficient of nitrogen absorption[58-60], stool fat content[60] and stool weight[60] have been docu-

Table 1 The ^{13}C-mixed triglycerides breath test according to Domínguez-Muñoz *et al*[44]

The patient fasts from midnight
Twenty minutes before the test, 10 mg of metoclopramide is ingested
A baseline breath sample is taken
At time 0, 250 mg of ^{13}C-mixed triglycerides mixed with 16 g of fat on a piece of toasted bread is ingested, together with a glass (200 mL) of water
Breath samples are taken every 15 (or 30) min for 6 h
Finally, $^{13}CO_2/^{12}CO_2$ is measured in collected breath samples by mass spectrometry or isotope-selective nondispersive infrared spectrometry

Table 2 Pancreatic enzyme replacement therapy: How we do it

PERT is started at 50000 lipase units per main meal and 25000 lipase units per snack
The basic concepts of the pathophysiology of PEI and how PERT works are explained to the patient. It is emphasized that PERT should be taken with meals and that the dose should be adjusted to the fat content of the meal
If maldigestion persists, proton pump inhibitors can be added, and the dose is increased to 80000 lipase units per main meal and 40000 lipase units per snack
If PERT is still ineffective, despite the optimization described above, small intestinal bacterial overgrowth is considered, and the evidence for a diagnosis of PEI is revised

PEI: Pancreatic exocrine insufficiency; PERT: Pancreatic enzyme replacement therapy.

mented. Two of these trials have also reported results from open-label extension periods of 6 and 12 mo[62,69]. Continuous improvement during treatment, with a steady-state reached at week 13, was observed for most symptom variables[62]. In these clinical trials, PERT has been well tolerated, and no serious adverse events have been reported. Fibrosing colonopathy is the only serious complication that has been associated with PERT. In the vast majority of reported cases, this rare condition has been observed in patients with cystic fibrosis using high doses of PERT[70]. The pathophysiology of this condition is unknown, but factors related to cystic fibrosis disease per se, the dosing of PERT and possibly agents in the enteric coating of the pancrelipase preparations may play a role[71].

There are no studies investigating the long-term effects of PERT on morbidity and mortality from PEI, which should be kept in mind if patients with no or minimal symptoms of PEI are considered for PERT.

Dosing of pancreatic enzyme replacement therapy

The concept of PERT is to induce a lipolytic capacity that corresponds to the amount of ingested fat at every meal. Therefore, higher doses are necessary for large, high-fat meals, and lower doses are sufficient for snacks and lean meals. The optimal dose of PERT in CP has not been investigated systematically in clinical trials. Recommendations from different national societies range from 20-40000 lipase units per main meal, as recommended by the German Society of Digestive and Metabolic Diseases[72], to 25-40000 lipase units per main meal, as recommended by the Australasian Pancreatic Club[18] and the Italian Association for the Study of the Pancreas[19], to 40-50000 lipase units per mail mean, as recommended by The Spanish Pancreatic Club[68]. In general, half of the dose is recommended for snacks and minor meals. Recent randomized clinical trials have used higher doses (72000 USP units[59] and 80000 Ph.Eur.U units[60] per main meal). It is noteworthy that even in a study using 80000 lipase units per main meal, only 26% of the patients had a normalized coefficient of fat absorption at the end of the 51-wk open-label extension of the study[62].

There is no consensus on the definition of treatment success in PERT. If the elimination of maldigestion is accepted as the aim of the treatment, fecal fat quantification or an indirect test of maldigestion, such as the ^{13}C-MTG breath test, would be the most appropriate examination for verifying treatment success. However, this approach is rarely feasible in clinical practice. Most guidelines recommend a reevaluation of symptoms and weight and a reevaluation of serum tests of malnutrition.

In fact, it is highly likely that many patients with PEI today are receiving PERT that is suboptimal. A recent study from the Netherlands has indicated that as many as 70% of patients with CP report steatorrhea-related symptoms despite PERT[73] and that persistent deficits in blood nutritional parameters despite PERT are common in PEI[15,64]. What measures can be taken to optimize the result of PERT? First, it is of utmost importance to ensure that the patient is taking the prescribed dose correctly. Capsules should be administered with meals (as opposed to before or after) for optimal effect[74]. If signs or symptoms of maldigestion persist, the PERT dose can be increased, and proton pump inhibitors can be added[44,75,76]. The rational for adjuvant treatment with proton pump inhibitors is that bicarbonate secretion is impaired in CP, resulting in insufficient buffering of the gastric chyme when it enters the small bowel. This phenomenon may in turn compromise the effect of PERT because lipase is rapidly degraded at a low pH and because enzyme release from microspheres is pH dependent. If PERT is ineffective despite an increased dose and adjuvant treatment with PPI, the diagnosis of PEI should be revised, and possible coexisting and/or alternative reasons for maldigestion, such as small intestinal bacterial overgrowth, should be considered. A summary of PERT concepts in clinical practice is presented in Table 2.

In conclusion, PEI is a state of maldigestion that is the result of a reduction of pancreatic enzyme activity in the intestinal lumen to a level that is below the threshold required to maintain normal digestion. CP is the most common cause of PEI, but several other pancreatic and extrapancreatic diseases can lead to PEI. The diagnosis of PEI is best established by tests that directly measure digestion, such as fecal fat quantification or the ^{13}C-MTG breath test. If these tests are not available, clinical and biochemical signs of malnutrition; pancreatic imaging findings; and tests that measure pancreatic secretion, such as the FE-1 assay, can be used to estimate the probability of PEI. The treatment of PEI relies on the elimination of risk factors for disease progression, such as smoking and alcohol consumption; consultation with a dietitian; PERT; and a systematic follow-up of the treatment effect on nutritional status and symptoms. If required, PERT can be optimized by dose augmentation and the addition of proton pump inhibitors.

REFERENCES

1 **DiMagno EP**, Go VL, Summerskill WH. Relations between pancreatic enzyme ouputs and malabsorption in severe pancreatic insufficiency. *N Engl J Med* 1973; **288**: 813-815 [PMID: 4693931 DOI: 10.1056/NEJM197304192881603]

2 **Leeds JS**, Hopper AD, Hurlstone DP, Edwards SJ, McAlindon ME, Lobo AJ, Donnelly MT, Morley S, Sanders DS. Is exocrine pancreatic insufficiency in adult coeliac disease a cause of persisting symptoms? *Aliment Pharmacol Ther* 2007; **25**: 265-271 [PMID: 17269988 DOI: 10.1111/j.1365-2036.2006.03206.x]

3 **Kingham JG**, Levison DA, Fairclough PD. Diarrhoea and reversible enteropathy in Zollinger-Ellison syndrome. *Lancet* 1981; **2**: 610-612 [PMID: 6116088]

4 **Domínguez-Muñoz JE**. Pancreatic enzyme replacement therapy: exocrine pancreatic insufficiency after gastrointestinal surgery. *HPB* (Oxford) 2009; **11** Suppl 3: 3-6 [PMID: 20495625 DOI: 10.1111/j.1477-2574.2009.00132.x]

5 **Keller J**, Layer P. Human pancreatic exocrine response to nutrients in health and disease. *Gut* 2005; **54** Suppl 6: vi1-v28 [PMID: 15951527]

6 **Anagnostides A**, Chadwick VS, Selden AC, Maton PN. Sham feeding and pancreatic secretion. Evidence for direct vagal stimulation of enzyme output. *Gastroenterology* 1984; **87**: 109-114 [PMID: 6724252]

7 **White TT**, Mcalexander RA, Magee DF. The effect of gastric distension on duodenal aspirates in man. *Gastroenterology* 1963; **44**: 48-51 [PMID: 14000362]

8 **Watanabe S**, Shiratori K, Takeuchi T, Chey WY, You CH, Chang TM. Release of cholecystokinin and exocrine pancreatic secretion in response to an elemental diet in human subjects. *Dig Dis Sci* 1986; **31**: 919-924 [PMID: 3731983]

9 **Pandol SJ**. Neurohumoral control of exocrine pancreatic secretion. *Curr Opin Gastroenterol* 2003; **19**: 443-446 [PMID: 15703586]

10 **Schaffalitzky de Muckadell OB**, Fahrenkrug J, Nielsen J, Westphall I, Worning H. Meal-stimulated secretin release in man: effect of acid and bile. *Scand J Gastroenterol* 1981; **16**: 981-988 [PMID: 7336139]

11 **Schaffalitzky de Muckadell OB**, Fahrenkrug J, Watt-Boolsen S, Worning H. Pancreatic response and plasma secretin concentration during infusion of low dose secretin in man. *Scand J Gastroenterol* 1978; **13**: 305-311 [PMID: 755275]

12 **DiMagno EP**, Malagelada JR, Go VL, Moertel CG. Fate of orally ingested enzymes in pancreatic insufficiency. Comparison of two dosage schedules. *N Engl J Med* 1977; **296**: 1318-1322 [PMID: 16213 DOI: 10.1056/NEJM197706092962304]

13 **Layer P**, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology* 1994; **107**: 1481-1487 [PMID: 7926511]

14 **Lindkvist B**, Domínguez-Muñoz JE, Luaces-Regueira M, Castiñeiras-Alvariño M, Nieto-Garcia L, Iglesias-Garcia J. Serum nutritional markers for prediction of pancreatic exocrine insufficiency in chronic pancreatitis. *Pancreatology* 2012; **12**: 305-310 [PMID: 22898630 DOI: 10.1016/j.pan.2012.04.006]

15 **Sikkens EC**, Cahen DL, Koch AD, Braat H, Poley JW, Kuipers EJ, Bruno MJ. The prevalence of fat-soluble vitamin deficiencies and a decreased bone mass in patients with chronic pancreatitis. *Pancreatology* 2013; **13**: 238-242 [PMID: 23719594 DOI: 10.1016/j.pan.2013.02.008]

16 **Tignor AS**, Wu BU, Whitlock TL, Lopez R, Repas K, Banks PA, Conwell D. High prevalence of low-trauma fracture in chronic pancreatitis. *Am J Gastroenterol* 2010; **105**: 2680-2686 [PMID: 20736937 DOI: 10.1038/ajg.2010.325]

17 **Van De Kamer JH**, Ten Bokkel Huinink H, Weyers HA. Rapid method for the determination of fat in feces. *J Biol Chem* 1949; **177**: 347-355 [PMID: 18107439]

18 **Toouli J**, Biankin AV, Oliver MR, Pearce CB, Wilson JS, Wray NH. Management of pancreatic exocrine insufficiency: Australasian Pancreatic Club recommendations. *Med J Aust* 2010; **193**: 461-467 [PMID: 20955123]

19 **Frulloni L**, Falconi M, Gabbrielli A, Gaia E, Graziani R, Pezzilli R, Uomo G, Andriulli A, Balzano G, Benini L, Calculli L, Campra D, Capurso G, Cavestro GM, De Angelis C, Ghezzo L, Manfredi R, Malesci A, Mariani A, Mutignani M, Ventrucci M, Zamboni G, Amodio A, Vantini I, Bassi C, Delle Fave G, Frulloni L, Vantini I, Falconi M, Frulloni L, Gabbrielli A, Graziani R, Pezzilli R, Capurso IV, Cavestro GM, De Angelis C, Falconi M, Gaia E, Ghezzo L, Gabbrielli A, Graziani R, Manfredi R, Malesci A, Mariani A, Mutignani M, Pezzilli R, Uomo G, Ventrucci M, Zamboni G, Vantini I, Magarini F, Albarello L, Alfieri S, Amodio A, Andriulli A, Anti M, Arcidiacono P, Baiocchi L, Balzano G, Benini L, Berretti D, Boraschi P, Buscarini E, Calculli L, Carroccio A, Campra D, Celebrano MR, Capurso G, Casadei R, Cavestro GM, Chilovi F, Conigliaro R, Dall'Oglio L, De Angelis C, De Boni M, De Pretis G, Di Priolo S, Di Sebastiano PL, Doglietto GB, Falconi M, Filauro M, Frieri G, Frulloni L, Fuini A, Gaia E, Ghezzo L, Gabbrielli A, Graziani R, Loriga P, Macarri G, Manes G, Manfredi R, Malesci A, Mariani A, Massucco P, Milani S, Mutignani M, Pasquali C, Pederzoli P, Pezzilli R, Pietrangeli M, Rocca R, Russello D, Siquini W, Traina M, Uomo G, Veneroni L, Ventrucci M, Zilli M, Zamboni G. Italian consensus guidelines for chronic pancreatitis. *Dig Liver Dis* 2010; **42** Suppl 6: S381-S406 [PMID: 21078490 DOI: 10.1016/S1590-8658(10)60682-2]

20 **Hartmann D**, Felix K, Ehmann M, Schnölzer M, Fiedler S, Bogumil R, Büchler M, Friess H. Protein expression profiling reveals distinctive changes in serum proteins associated with chronic pancreatitis. *Pancreas* 2007; **35**: 334-342 [PMID: 18090239 DOI: 10.1097/mpa.0b013e3180cac723]

21 **Montalto G**, Soresi M, Carroccio A, Scafidi E, Barbagallo CM, Ippolito S, Notarbartolo A. Lipoproteins and chronic pancreatitis. *Pancreas* 1994; **9**: 137-138 [PMID: 8108368]

22 **Sobral-Oliveira MB**, Faintuch J, Guarita DR, Oliveira CP, Carrilho FJ. Nutritional profile of asymptomatic alcoholic patients. *Arq Gastroenterol* 2011; **48**: 112-118 [PMID: 21709952]

23 **Schnitzler CM**, Mesquita JM, Shires R. Cortical and trabecular bone microarchitecture and turnover in alcohol-induced chronic pancreatitis: a histomorphometric study. *J Bone Miner*

Metab 2010; **28**: 456-467 [PMID: 20101423 DOI: 10.1007/s00774-009-0151-x]

24 **Kalvaria I**, Labadarios D, Shephard GS, Visser L, Marks IN. Biochemical vitamin E deficiency in chronic pancreatitis. *Int J Pancreatol* 1986; **1**: 119-128 [PMID: 3693979 DOI: 10.1007/BF02788444]

25 **Twersky Y**, Bank S. Nutritional deficiencies in chronic pancreatitis. *Gastroenterol Clin North Am* 1989; **18**: 543-565 [PMID: 2680966]

26 **Marotta F**, Labadarios D, Frazer L, Girdwood A, Marks IN. Fat-soluble vitamin concentration in chronic alcohol-induced pancreatitis. Relationship with steatorrhea. *Dig Dis Sci* 1994; **39**: 993-998 [PMID: 8174441]

27 **Glasbrenner B**, Malfertheiner P, Büchler M, Kuhn K, Ditschuneit H. Vitamin B12 and folic acid deficiency in chronic pancreatitis: a relevant disorder? *Klin Wochenschr* 1991; **69**: 168-172 [PMID: 2041378]

28 **Domínguez-Muñoz JE**, Alvarez-Castro A, Lariño-Noia J, Nieto L, Iglesias-García J. Endoscopic ultrasonography of the pancreas as an indirect method to predict pancreatic exocrine insufficiency in patients with chronic pancreatitis. *Pancreas* 2012; **41**: 724-728 [PMID: 22228053 DOI: 10.1097/MPA.0b013e31823b5978]

29 **Domínguez-Muñoz JE**, Manes G, Pieramico O, Büchler M, Malfertheiner P. Effect of pancreatic ductal and parenchymal changes on exocrine function in chronic pancreatitis. *Pancreas* 1995; **10**: 31-35 [PMID: 7899457]

30 **Catalano MF**, Sahai A, Levy M, Romagnuolo J, Wiersema M, Brugge W, Freeman M, Yamao K, Canto M, Hernandez LV. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. *Gastrointest Endosc* 2009; **69**: 1251-1261 [PMID: 19243769]

31 **Sziegoleit A**, Krause E, Klör HU, Kanacher L, Linder D. Elastase 1 and chymotrypsin B in pancreatic juice and feces. *Clin Biochem* 1989; **22**: 85-89 [PMID: 2720968]

32 **Löser C**, Möllgaard A, Fölsch UR. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut* 1996; **39**: 580-586 [PMID: 8944569]

33 **Leeds JS**, Oppong K, Sanders DS. The role of fecal elastase-1 in detecting exocrine pancreatic disease. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 405-415 [PMID: 21629239 DOI: 10.1038/nrgastro.2011.91]

34 **Stein J**, Jung M, Sziegoleit A, Zeuzem S, Caspary WF, Lembcke B. Immunoreactive elastase I: clinical evaluation of a new noninvasive test of pancreatic function. *Clin Chem* 1996; **42**: 222-226 [PMID: 8595714]

35 **Hardt PD**, Marzeion AM, Schnell-Kretschmer H, Wüsten O, Nalop J, Zekorn T, Klör HU. Fecal elastase 1 measurement compared with endoscopic retrograde cholangiopancreatography for the diagnosis of chronic pancreatitis. *Pancreas* 2002; **25**: e6-e9 [PMID: 12131782]

36 **Bilgin M**, Bilgin S, Balci NC, Momtahen AJ, Bilgin Y, Klör HU, Rau WS. Magnetic resonance imaging and magnetic resonance cholangiopancreatography findings compared with fecal elastase 1 measurement for the diagnosis of chronic pancreatitis. *Pancreas* 2008; **36**: e33-e39 [PMID: 18192870 DOI: 10.1097/mpa.0b013e318150e557]

37 **Benini L**, Amodio A, Campagnola P, Agugiaro F, Cristofori C, Micciolo R, Magro A, Gabbrielli A, Cabrini G, Moser L, Massella A, Vantini I, Frulloni L. Fecal elastase-1 is useful in the detection of steatorrhea in patients with pancreatic diseases but not after pancreatic resection. *Pancreatology* 2013; **13**: 38-42 [PMID: 23395568 DOI: 10.1016/j.pan.2012.11.307]

38 **Vantrappen GR**, Rutgeerts PJ, Ghoos YF, Hiele MI. Mixed triglyceride breath test: a noninvasive test of pancreatic lipase activity in the duodenum. *Gastroenterology* 1989; **96**: 1126-1134 [PMID: 2494097]

39 **Bożek M**, Jonderko K, Piłka M. On a refinement of the ^{13}C-mixed TAG breath test. *Br J Nutr* 2012; **107**: 211-217 [PMID: 21733305 DOI: 10.1017/S0007114511002881]

40 **Iglesias-Garcia J, Vilarino** M, Iglesias-Rey M, Lourido V, Dominguez-Munoz E. Accuracy of the optimized 13C-mixed triglyceride breath test for the diagnosis of steatorrhea in clinical practice. *Gastroenterology* 2003; **124** Supp 1: A631

41 **Boedeker C**, Goetze O, Pfaffenbach B, Luypaerts A, Geypens B, Adamek RJ. 13C mixed-triglyceride breath test: isotope selective non-dispersive infrared spectrometry in comparison with isotope ratio mass spectrometry in volunteers and patients with chronic pancreatitis. *Scand J Gastroenterol* 1999; **34**: 1153-1156 [PMID: 10582768]

42 **Löser C**, Brauer C, Aygen S, Hennemann O, Fölsch UR. Comparative clinical evaluation of the 13C-mixed triglyceride breath test as an indirect pancreatic function test. *Scand J Gastroenterol* 1998; **33**: 327-334 [PMID: 9548629]

43 **Keller J**, Brückel S, Jahr C, Layer P. A modified ^{13}C-mixed triglyceride breath test detects moderate pancreatic exocrine insufficiency. *Pancreas* 2011; **40**: 1201-1205 [PMID: 21705945 DOI: 10.1097/MPA.0b013e318220ad98]

44 **Domínguez-Muñoz JE**, Iglesias-García J, Vilariño-Insua M, Iglesias-Rey M. 13C-mixed triglyceride breath test to assess oral enzyme substitution therapy in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2007; **5**: 484-488 [PMID: 17445754 DOI: 10.1016/j.cgh.2007.01.004]

45 **Burton P**, Evans DG, Harper AA, Howath T, Oleesky S, Scott JE, Varley H. A test of pancreatic function in man based on the analysis of duodenal contents after administration of secretin and pancreozymin. *Gut* 1960; **1**: 111-124 [PMID: 13806339]

46 **Borgstrom B**, Dahlqvist A, Lundh G. On the site of absorption of fat from the human small intestine. *Gut* 1962; **3**: 315-317 [PMID: 14013961]

47 **Stevens T**, Conwell DL, Zuccaro G, Van Lente F, Lopez R, Purich E, Fein S. A prospective crossover study comparing secretin-stimulated endoscopic and Dreiling tube pancreatic function testing in patients evaluated for chronic pancreatitis. *Gastrointest Endosc* 2008; **67**: 458-466 [PMID: 18294508 DOI: 10.1016/j.gie.2007.07.028]

48 **Stevens T**, Conwell DL, Zuccaro G, Van Lente F, Purich E, Khandwala F, Fein S. A randomized crossover study of secretin-stimulated endoscopic and dreiling tube pancreatic function test methods in healthy subjects. *Am J Gastroenterol* 2006; **101**: 351-355 [PMID: 16454842]

49 **Schneider AR**, Hammerstingl R, Heller M, Povse N, Murzynski L, Vogl TJ, Caspary WF, Stein J. Does secretin-stimulated MRCP predict exocrine pancreatic insufficiency?: A comparison with noninvasive exocrine pancreatic function tests. *J Clin Gastroenterol* 2006; **40**: 851-855 [PMID: 17016144 DOI: 10.1097/01.mcg.0000225652.00308.a2]

50 **Balci NC**, Smith A, Momtahen AJ, Alkaade S, Fattahi R, Tariq S, Burton F. MRI and S-MRCP findings in patients with suspected chronic pancreatitis: correlation with endoscopic pancreatic function testing (ePFT). *J Magn Reson Imaging* 2010; **31**: 601-606 [PMID: 20187202 DOI: 10.1002/jmri.22085]

51 **Domínguez-Muñoz JE**. Pancreatic exocrine insufficiency: diagnosis and treatment. *J Gastroenterol Hepatol* 2011; **26** Suppl 2: 12-16 [PMID: 21323992 DOI: 10.1111/j.1440-1746.2010.06600.x]

52 **Gullo L**, Barbara L, Labò G. Effect of cessation of alcohol use on the course of pancreatic dysfunction in alcoholic pancreatitis. *Gastroenterology* 1988; **95**: 1063-1068 [PMID: 3410221]

53 **Yadav D**, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1252-1261 [PMID: 23622135 DOI: 10.1053/j.gastro.2013.01.068]

54 **Law R**, Parsi M, Lopez R, Zuccaro G, Stevens T. Cigarette smoking is independently associated with chronic pancreatitis. *Pancreatology* 2010; **10**: 54-59 [PMID: 20332662 DOI: 10.1159/000225927]

55 **Maisonneuve P**, Lowenfels AB, Müllhaupt B, Cavallini G, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L, Frulloni L, Ammann RW. Cigarette smoking accelerates progression of alcoholic chronic pancreatitis. *Gut*

2005; **54**: 510-514 [PMID: 15753536]

56 **Löhr JM**, Hummel FM, Pirilis KT, Steinkamp G, Körner A, Henniges F. Properties of different pancreatin preparations used in pancreatic exocrine insufficiency. *Eur J Gastroenterol Hepatol* 2009; **21**: 1024-1031 [PMID: 19352190 DOI: 10.1097/MEG.0b013e328328f414]

57 **Safdi M**, Bekal PK, Martin S, Saeed ZA, Burton F, Toskes PP. The effects of oral pancreatic enzymes (Creon 10 capsule) on steatorrhea: a multicenter, placebo-controlled, parallel group trial in subjects with chronic pancreatitis. *Pancreas* 2006; **33**: 156-162 [PMID: 16868481 DOI: 10.1097/01.mpa.0000226884.32957.5e]

58 **Toskes PP**, Secci A, Thieroff-Ekerdt R. Efficacy of a novel pancreatic enzyme product, EUR-1008 (Zenpep), in patients with exocrine pancreatic insufficiency due to chronic pancreatitis. *Pancreas* 2011; **40**: 376-382 [PMID: 21343835 DOI: 10.1097/MPA.0b013e31820b971c]

59 **Whitcomb DC**, Lehman GA, Vasileva G, Malecka-Panas E, Gubergrits N, Shen Y, Sander-Struckmeier S, Caras S. Pancrelipase delayed-release capsules (CREON) for exocrine pancreatic insufficiency due to chronic pancreatitis or pancreatic surgery: A double-blind randomized trial. *Am J Gastroenterol* 2010; **105**: 2276-2286 [PMID: 20502447 DOI: 10.1038/ajg.2010.201]

60 **Thorat V**, Reddy N, Bhatia S, Bapaye A, Rajkumar JS, Kini DD, Kalla MM, Ramesh H. Randomised clinical trial: the efficacy and safety of pancreatin enteric-coated minimicrospheres (Creon 40000 MMS) in patients with pancreatic exocrine insufficiency due to chronic pancreatitis--a double-blind, placebo-controlled study. *Aliment Pharmacol Ther* 2012; **36**: 426-436 [PMID: 22762290 DOI: 10.1111/j.1365-2036.2012.05202.x]

61 **Czakó L**, Takács T, Hegyi P, Prónai L, Tulassay Z, Lakner L, Döbrönte Z, Boda K, Lonovics J. Quality of life assessment after pancreatic enzyme replacement therapy in chronic pancreatitis. *Can J Gastroenterol* 2003; **17**: 597-603 [PMID: 14571298]

62 **Ramesh H**, Reddy N, Bhatia S, Rajkumar JS, Bapaye A, Kini D, Kalla M, Thorat V. A 51-week, open-label clinical trial in India to assess the efficacy and safety of pancreatin 40000 enteric-coated minimicrospheres in patients with pancreatic exocrine insufficiency due to chronic pancreatitis. *Pancreatology* 2013; **13**: 133-139 [PMID: 23561971 DOI: 10.1016/j.pan.2013.01.009]

63 **Dumasy V**, Delhaye M, Cotton F, Deviere J. Fat malabsorption screening in chronic pancreatitis. *Am J Gastroenterol* 2004; **99**: 1350-1354 [PMID: 15233677 DOI: 10.1111/j.1572-0241.2004.30661.x]

64 **Domínguez-Muñoz JE**, Iglesias-García J. Oral pancreatic enzyme substitution therapy in chronic pancreatitis: is clinical response an appropriate marker for evaluation of therapeutic efficacy? *JOP* 2010; **11**: 158-162 [PMID: 20208327]

65 **Domínguez-Muñoz JE**. Chronic pancreatitis and persistent steatorrhea: what is the correct dose of enzymes? *Clin Gastroenterol Hepatol* 2011; **9**: 541-546 [PMID: 21377551 DOI: 10.1016/j.cgh.2011.02.027]

66 **Morán CE**, Sosa EG, Martinez SM, Geldern P, Messina D, Russo A, Boerr L, Bai JC. Bone mineral density in patients with pancreatic insufficiency and steatorrhea. *Am J Gastroenterol* 1997; **92**: 867-871 [PMID: 9149203]

67 **Haaber AB**, Rosenfalck AM, Hansen B, Hilsted J, Larsen S. Bone mineral metabolism, bone mineral density, and body composition in patients with chronic pancreatitis and pancreatic exocrine insufficiency. *Int J Pancreatol* 2000; **27**: 21-27 [PMID: 10811020]

68 **de-Madaria E**, Abad-González A, Aparicio JR, Aparisi L, Boadas J, Boix E, de-Las-Heras G, Domínguez-Muñoz E, Farré A, Fernández-Cruz L, Gómez L, Iglesias-García J, García-Malpartida K, Guarner L, Lariño-Noia J, Lluís F, López A, Molero X, Moreno-Pérez O, Navarro S, Palazón JM, Pérez-Mateo M, Sabater L, Sastre Y, Vaquero EC, Martínez J. The Spanish Pancreatic Club's recommendations for the diagnosis and treatment of chronic pancreatitis: part 2 (treatment). *Pancreatology* 2013; **13**: 18-28 [PMID: 23395565 DOI: 10.1016/j.pan.2012.11.310]

69 **Gubergrits N**, Malecka-Panas E, Lehman GA, Vasileva G, Shen Y, Sander-Struckmeier S, Caras S, Whitcomb DC. A 6-month, open-label clinical trial of pancrelipase delayed-release capsules (Creon) in patients with exocrine pancreatic insufficiency due to chronic pancreatitis or pancreatic surgery. *Aliment Pharmacol Ther* 2011; **33**: 1152-1161 [PMID: 21418260 DOI: 10.1111/j.1365-2036.2011.04631.x]

70 **FitzSimmons SC**, Burkhart GA, Borowitz D, Grand RJ, Hammerstrom T, Durie PR, Lloyd-Still JD, Lowenfels AB. High-dose pancreatic-enzyme supplements and fibrosing colonopathy in children with cystic fibrosis. *N Engl J Med* 1997; **336**: 1283-1289 [PMID: 9113931 DOI: 10.1056/NEJM199705013361803]

71 **Nakajima K**, Oshida H, Muneyuki T, Kakei M. Pancrelipase: an evidence-based review of its use for treating pancreatic exocrine insufficiency. *Core Evid* 2012; **7**: 77-91 [PMID: 22936895 DOI: 10.2147/CE.S26705]

72 **Hoffmeister A**, Mayerle J, Beglinger C, Büchler MW, Bufler P, Dathe K, Fölsch UR, Friess H, Izbicki J, Kahl S, Klar E, Keller J, Knoefel WT, Layer P, Loehr M, Meier R, Riemann JF, Rünzi M, Schmid RM, Schreyer A, Tribl B, Werner J, Witt H, Mössner J, Lerch MM. [S3-Consensus guidelines on definition, etiology, diagnosis and medical, endoscopic and surgical management of chronic pancreatitis German Society of Digestive and Metabolic Diseases (DGVS)]. *Z Gastroenterol* 2012; **50**: 1176-1224 [PMID: 23150111 DOI: 10.1055/s-0032-1325479]

73 **Sikkens EC**, Cahen DL, van Eijck C, Kuipers EJ, Bruno MJ. Patients with exocrine insufficiency due to chronic pancreatitis are undertreated: a Dutch national survey. *Pancreatology* 2012; **12**: 71-73 [PMID: 22487479 DOI: 10.1016/j.pan.2011.12.010]

74 **Domínguez-Muñoz JE**, Iglesias-García J, Iglesias-Rey M, Figueiras A, Vilariño-Insua M. Effect of the administration schedule on the therapeutic efficacy of oral pancreatic enzyme supplements in patients with exocrine pancreatic insufficiency: a randomized, three-way crossover study. *Aliment Pharmacol Ther* 2005; **21**: 993-1000 [PMID: 15813835 DOI: 10.1111/j.1365-2036.2005.02390.x]

75 **Bruno MJ**, Rauws EA, Hoek FJ, Tytgat GN. Comparative effects of adjuvant cimetidine and omeprazole during pancreatic enzyme replacement therapy. *Dig Dis Sci* 1994; **39**: 988-992 [PMID: 8174440]

76 **Regan PT**, Malagelada JR, DiMagno EP, Glanzman SL, Go VL. Comparative effects of antacids, cimetidine and enteric coating on the therapeutic response to oral enzymes in severe pancreatic insufficiency. *N Engl J Med* 1977; **297**: 854-858 [PMID: 20572 DOI: 10.1056/NEJM197710202971603]

P- Reviewers: Du YQ, Fernandez-Zapico ME, Wassef W, Zhang JX **S- Editor**: Wen LL **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7267

World J Gastroenterol 2013 November 14; 19(42): 7267-7275
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Nutrition in chronic pancreatitis

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Received: June 20, 2013 **Revised:** September 24, 2013
Accepted: October 19, 2013
Published online: November 14, 2013

Abstract

The pancreas is a major player in nutrient digestion. In chronic pancreatitis both exocrine and endocrine insufficiency may develop leading to malnutrition over time. Maldigestion is often a late complication of chronic pancreatic and depends on the severity of the underlying disease. The severity of malnutrition is correlated with two major factors: (1) malabsorption and depletion of nutrients (*e.g.*, alcoholism and pain) causes impaired nutritional status; and (2) increased metabolic activity due to the severity of the disease. Nutritional deficiencies negatively affect outcome if they are not treated. Nutritional assessment and the clinical severity of the disease are important for planning any nutritional intervention. Good nutritional practice includes screening to identify patients at risk, followed by a thoroughly nutritional assessment and nutrition plan for risk patients. Treatment should be multidisciplinary and the mainstay of treatment is abstinence from alcohol, pain treatment, dietary modifications and pancreatic enzyme supplementation. To achieve energy-end protein requirements, oral supplementation might be beneficial. Enteral nutrition may be used when patients do not have sufficient calorie intake as in pylero-duodenal-stenosis, inflammation or prior to surgery and can be necessary if weight loss continues. Parenteral nutrition is very seldom used in patients with chronic pancreatitis and should only be used in case of GI-tract obstruction or as a supplement to enteral nutrition.

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Key words: Chronic pancreatitis; Malnutrition; Nutritional risk; Malabsorption; Nutritional risk screening; Metabolism; Nutritional assessment; Nutrition therapy

Core tip: The pancreas is a major player in nutrient digestion and malnutrition is frequently found but is often neglected. The severity of malnutrition is correlated with malabsorption and depletion of nutrients (*e.g.*, alcoholism and pain) that causes impaired nutritional status and increased metabolic activity due to the severity of the disease. Good nutritional practice includes screening to identify patients at nutritional risk, followed by a thoroughly nutritional assessment and nutrition plan for risk patients. Treatment should be multidisciplinary and the mainstay of treatment is abstinence from alcohol, pain treatment, dietary modifications and pancreatic enzyme supplementation.

Rasmussen HH, Irtun Ø, Olesen SS, Drewes AM, Holst M. Nutrition in chronic pancreatitis. *World J Gastroenterol* 2013; 19(42): 7267-7275 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7267.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7267

INTRODUCTION

Chronic pancreatitis (CP) is an inflammatory disorder that causes irreversible anatomical changes and damage, including infiltration of inflammatory cells, fibrosis and calcification of the pancreas with destruction of the glandular structure and thereby affects normal digestion and absorption of nutrients.

Maldigestion is often a late complication of CP and depends on the severity of the underlying disease. The medium latency between onset of first symptoms and signs of maldigestion is about 8-9 years in alcoholic CP and more than 15 years in idiopathic non-alcoholic pancreatitis. Nutrient deficiencies are common in CP, driven by many risk factors including malabsorption, diabetes and, in alcoholic CP, alcoholism. However, deficiencies are frequently overlooked, leading to malnutrition[1,2].

The aim of this article is to discuss the definition of malnutrition and good nutritional practice in patients with CP.

DEFINITION OF MALNUTRITION AND GOOD NUTRITIONAL PRACTICE

Generally about 20%-50% of all patients in hospital are found at risk of undernutrition, dependent on definition, clinical setting and screening tool amended. A large part of these patients are at nutritional risk when admitted to hospital and in the majority of these, undernutrition develops negatively during hospital stay[3,4].

This can be prevented if special attention is paid to nutritional care of patients. Routine identification is paramount as a first stage in a patient's care in order to identify at-risk patients, with a view to providing nutrition support if necessary[5,6].

Good nutritional practice for the patient starts by nutrition screening. The European Society for Clinical Nutrition and Metabolism (ESPEN) Guidelines for Nutrition Screening recommend a continuity of issues to be considered in all patients admitted to hospital (Figure 1)[6]. (1) Initially on admission, a simple nutritional screening is to be done, to identify patients at actual nutritional risk; (2) Subsequently for patients at nutritional risk, a thorough nutritional assessment is to be completed; (3) This stage leads to an individual evaluation of nutritional requirements, and a plan for nutrition therapy and care; and (4) Monitoring and defining of targeted outcome should be structured in order to reconsider therapy and care-planning. Finally, communication around results of screening, assessment, plan and monitoring should be communicated to other health care professionals, when the patient is transferred, either back to the community or to another institution[7-9].

In order to give priority to nutritional intervention for relevant patients, nutrition screening methods have been developed and validated. These screening methods regard nutritional status and acute disease. However, there is still no clear consensus agreement on a uniform definition of undernutrition or agreement for a gold standard method of screening and identification of undernutrition[10]. This also applies for patients with CP and malnutrition which has been offered numerous definitions. Simply stated, malnutrition is a sub-optimal nutrient status appearing as a consequence of nutrient deficiencies, which change body composition and functional status. However, this definition neglects the numerous causes of malnutrition. An international guideline committee has recently proposed an aetiology-based approach that incorporates a current understanding of the inflammatory response, as seen in CP and many other patients. The committee proposed the following nomenclature for nutrition diagnosis in adults in the clinical practice setting: "Starvation-related malnutrition", for chronic starvation without inflammation *i.e.*, anorexia nervosa, "chronic disease-related malnutrition", when inflammation is chronic and of mild to moderate degree *i.e.*, CP, and "acute disease or injury-related malnutrition", *i.e.*, CP with a serious complication in the intensive ward, that is, when inflammation is acute and of severe degree[11,12].

The causes of malnutrition in patients are thus included in its definition. As such, screening tools, which neglect to include relevant disease related parameters such as chronic or acute inflammation, may be less efficacious in identifying malnutrition risk.

Nutritional screening should be a simple and rapid process, which can be carried out by busy admitting nursing and medical staff. Most screening tools address four basic questions: recent weight loss, recent food intake, current body mass index and disease severity or some other measure of predicting risk of malnutrition. In 2003, ESPEN published guidelines for nutrition screening in the community, in the hospital and among elderly in institutions. The Nutrition Risk Screening 2002 (NRS 2002) seems to be the best validated screening tool, in terms of predictive validity, *i.e.*, that clinical outcome improves when patients identified to be at risk are treated, however the evidence is sparse[3,5,6,9].

For adult patients in hospital it is thus suggested to use the NRS 2002 (Figure 2)[9]. A score equal to or greater than 3 generates a nutrition plan in all cases. If the patient is at risk, but metabolic or functional problems prevent a standard plan being carried out, or if there is doubt as to whether the patient is at risk, a referral should be made to an expert or expert team for a more detailed assessment.

HOW DOES CP INFLUENCE NUTRITIONAL STATUS AND METABOLISM?

According to the definition, the severity of malnutrition is correlated with two major factors: depletion of nutrients (alcoholism and pain) and malabsorption causes impaired nutritional status and increased metabolic activity due to the inflammatory component of CP (severity of disease). Generally patients at nutritional risk have an increased number of complications and a poorer outcome, but specific studies investigating this issue in CP are however not available[2,13,14].

A persistent alcohol intake, pain after a meal and maldigestion are the main causes of weight loss, and weight loss is strongly associated with maldigestion of fat[1,14,15].

It is widely acknowledged that patients with CP are

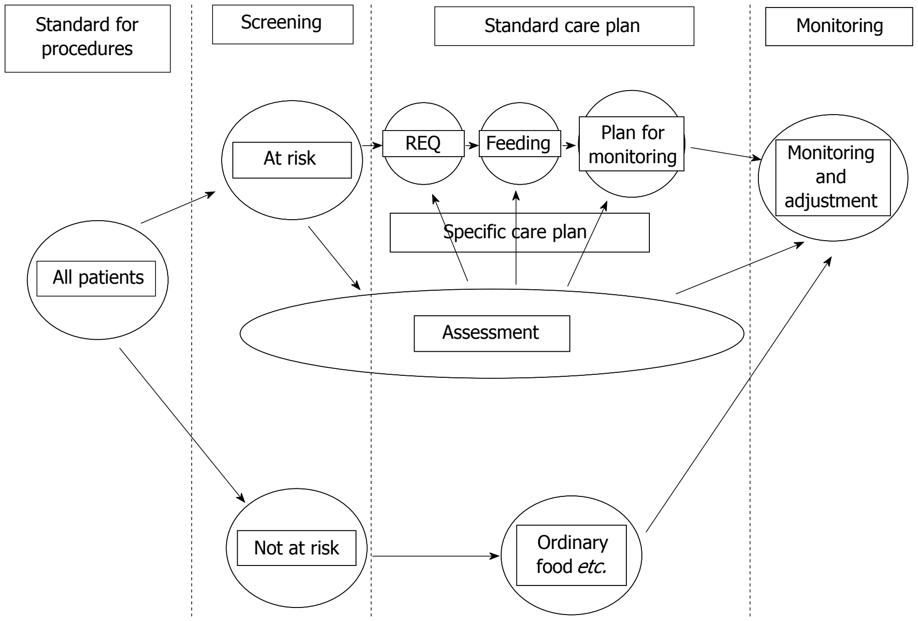

Figure 1 The nutritional care process including screening, plan and monitoring according to the European Society for Clinical Nutrition and Metabolism guideline[6]. REQ: Requirement.

often undernourished; however few studies have assessed this. In a medical rehabilitation clinic setting, 32% had a BMI < 20 kg/m^2; 57% chronic diarrhea and 24% steatorrhea[2].

Others have found that patients are malnourished prior to surgery and that the problem remains after surgery in a substantial proportion of patients with CP[16]. Furthermore, lean body mass and fat mass are found to be decreased both in patients with or without residual pancreatic function[17]. Decreased lean body mass may lead to decreased functional capacity as found in 34% of patients with moderate to severe weight loss. This also affected quality of life[18]. In this study fatigue, found in 46%, also had a major impact on quality of life. In a cohort of ambulatory patients with CP (60 patients), we found 28% at nutritional risk. These patients had a lower fat- and muscle mass and a tendency to lower handgrip strength. Furthermore, handgrip strength was associated with muscle- and fat mass[19].

It has been found that 30%-50% of patients with CP have increased resting energy expenditure[20]. We found no difference between estimated (Harris-Benedict) and measured resting energy expenditure (indirect calorimetric) in a cohort of ambulatory CP patients. However, when adjusted for fat free mass and height, we found higher resting energy expenditure (around 20%) in patients with low BMI (< 20 kg/m^2)[19].

Substrate metabolism during chronic pancreatitis

Daily intake of carbohydrates is about 300 grams corresponding to about half the caloric intake per day. About half of the caloric intake is carbohydrates and 30% are sucrose. Pancreatic alfa-amylase is the only enzyme for carbohydrate digestion that is produced by the pancreas. The final digestion of sugars take place at the brushborder of enterocytes where a range of disaccharidases produce the three sugars glucose, galactose and fructose that can be absorbed. In exocrine pancreatic insufficiency, carbohydrate digestion is maintained for a long time by salivary amylase and brush-border oligosaccharidoses. The loss of endocrine function leads to glucose intolerance in 40%-90% of patients with severe CP. Furthermore, in 20%-30% of patients an insulin-dependent diabetes develops associated with impaired glucagon and pancreatic polypeptide regulation (discussed in other article).

Daily protein intake in the Western world lies between 70 and 100 g and gastrointestinal secretions contain further 50-60 g of proteins per day. Protein digestion is initiated by intragastric proteolytic activity and continued by intestinal brush-border peptidases. Luminal proteolytic activity is maintained even in the absence of pancreatic peptidases and azotorrhea is therefore a very late and rare symptom in CP[21]. Trypsinogen is the inactive precursor for trypsin, which is the key enzyme for activation of all proteolytic enzymes in the duodenum. Trypsinogen becomes activated by enteropeptidase that is secreted by the brush-border of duodenal enterocytes. Proelastase is produced by the pancreas and belong to the chymotrypsin-like elastase family (elastase-2A, elastase-2B, elastase-3A, and elastase-3B). The elastase-3A isoform can be measured and quantified by the faecal elastase test. About 40% of proteins are digested to free amino acids. Brush border peptidases continue the digestion of peptides longer than three amino acids. Dipeptides, tripeptides, and free amino acids are then absorbed by the enterocytes by different transport mechanisms.

In Western diet up to 40% of the daily caloric intake derives from lipids, although 30% is recommended. Lipids are insoluble in water and need to be transferred to water-soluble micelles formed by bile acids, phospho-

	Step 1: Initial screening	Yes	No
1	Is BMI < 20.5 kg/m^2?		
2	Has the patient lost weight within the last 3 mo?		
3	Has the patient had a reduced dietary intake in the last week?		
4	Is the patient severely ill? (*e.g.*, in intensive therapy)		
Yes: If the answer is 'Yes' to any question, the screening in Step 2 is performed.			
No: If the answer is 'No' to all questions, the patient is re-screened at weekly intervals. If the patient *e.g.*, is scheduled for at major operation, a preventive nutritional care plan is considered to avoid the associated risk status.			

Step 2: Final screening			
Impaired nutritional status		Severity of disease (approximately increase in requirements)	
Absent Score 0	Normal nutritional status A	Absent Score 0	Normal nutritional requirements
Mild Score 1	Wt loss > 5% in 3 mo or Food intake below 50-75% of normal requirement in preceding week.	Mild Score 1	Hip fracture[1] Chronic patients, in particular with acute complications: cirrhosis[1], chronic obstructive pulmonary disease[1]. Chronic hemodialysis, diabetes, oncology.
Moderate Score 2	Wt loss > 5% in 2 mo or BMI 18.5-20.5 + impaired gen. condition or Food intake 25%-50% of normal requirement in preceding week	Moderate Score 2	Major abdominal surgery[1] Stroke[1] Severe pneumonia, hematologic malignancy.
Severe Score 3	Wt loss > 5% in 1 month (> 15% in 3 mo) or BMI > 18.5 + impaired general condition or Food intake 0-25% of normal requirement in preceding week	Severe Score 3	Head injury[1] Bone marrow transplantation Intensive care patients (APACHE >10).
Score:	+	Score:	= Total score:
Age	if ⩾ 70 yr: Add 1 to total score above		= Age-adjusted total score:
Score ⩾ 3: The patient is nutritionally at-risk and a nutritional care plan is initiated.			
Score < 3: Weekly rescreening of the patient. If the patient *e.g.*, is scheduled for a major operation, a preventive nutritional care plan is considered to avoid the associated risk status.			

NRS-2002 is bases on an interpretation of available randomized clinical trials.
[1]Indicates that a trial directly supports the categorization of patients with that diagnosis. Diagnoses shown in italics are based on the prototypes given below.
Nutritional risk is defined by the present nutritional status and risk of impairment of present status, due to increased requirements caused by stress metabolism of the clinical condition.

A nutritional care plan is indicated in all patients who are
(1) severely undernourished (score = 3)
or
(2) severely ill (score = 3)
or
(3) moderately undernourished + mildly ill (score 2 + 1)
or
(4) mildly undernourished + moderately ill (score 1 + 2)
Prototypes for severity of disease
Score = 1: A patient with chronic disease, admitted to hospital due to complications. The patient is weak but out of bed regularly.
Protein requirement is increased, but can be covered by oral or supplement in most cases.
Score = 2: A patient confined to bed due to illness, *e.g.*, following major abdominal surgery. Protein requirement is substantially increased, but can be covered, although artificial feeding is required in many cases.
Score = 3: A patient in intensive care with assisted ventilation etc. Protein requirement is increased and cannot be covered even by artificial feeding. Protein breakdown and nitrogen loss can be significantly attenuated.

Figure 2 Nutrition risk screening 2002[9]. BMI: Body mass index.

lipids, cholesterol and other products. This facilitates hydrolysis by lipase. In the stomach lingual lipase and gastric lipase hydrolyse triglycerides to glycerol and free fatty acids. Lipases from the gastric and salivary glands have a minor role in digestion of triglycerides and cannot compensate an insufficient pancreatic fat digestion. Thus, luminal lipid digestion within the small intestine depends on the action of pancreatic lipase and cofactors such as colipase and bile acids. There are no triglyceride-digestion enzyme systems within the intestinal brush-border membrane. Consequently, lipid digestion is decreased by insufficient lipase secretion and reduced luminal bile acid concentration. Because bicarbonate secretion is also diminished in CP and postprandial intraduodenal pH may fall < 4, luminal lipase degradation occurs more rapidly than that of other enzymes due to its greater instability. Gastric lipase only partly compensates for the lack of pancreatic lipase (discussed in other article).

Vitamin deficiency

A deficiency in vitamins A, D, E, K correlates with the severity of steatorrhoea in patients with CP, but may be caused by several different mechanisms including, suboptimal dietary intakes, increased losses, increased requirements, impaired binding of nutrients, antioxidant activity, and fat malabsorption. E vitamin deficiency may be seen more often than deficiencies of vitamin A, D, and K[22-24]. However, osteopathy (osteoporosis, osteomalacia, osteopenia) may occur in at least 25% of CP patients[22]. The adjusted hazard ratio (HR) for any fracture was 1.7 in patients with CP (95%CI: 1.6-1.8) in a recently Danish study[25]. Due to inadequate protease secretion by the pancreas, vitamin B12 deficiency can occur[26]. Zinc deficiency may be seen especially in association with diabetes[27]. Also, deficiencies in calcium, magnesium, thiamine and folic acids have been reported. The functional meaning of such deficiencies are how-

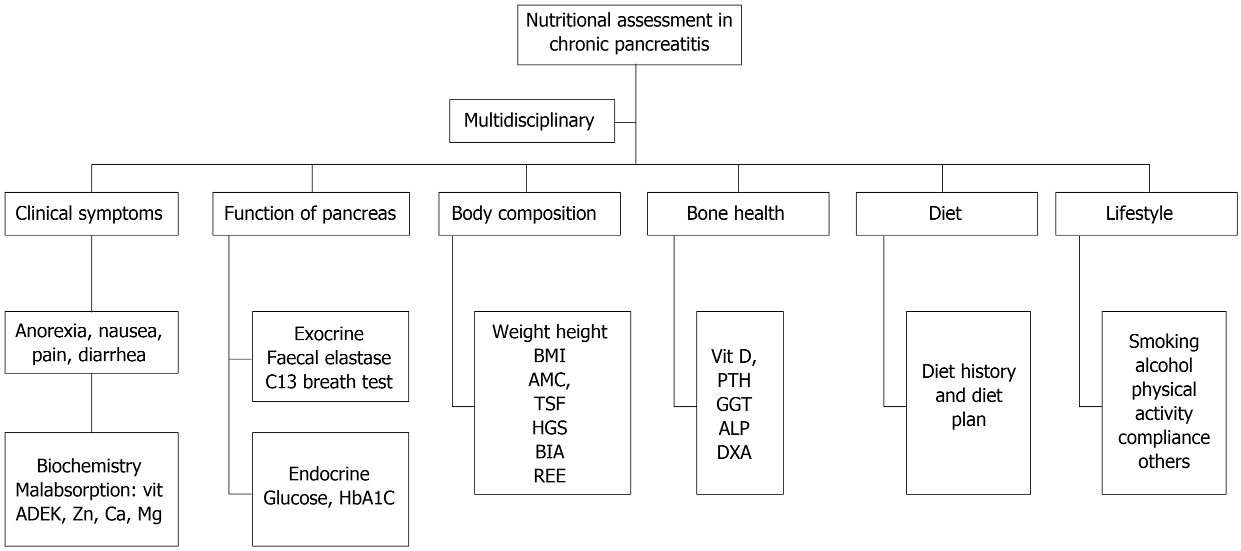
Nutritional assessment in chronic pancreatitis
Multidisciplinary
Clinical symptoms
Function of pancreas
Body composition
Bone health
Diet
Lifestyle
Anorexia, nausea, pain, diarrhea
Biochemistry Malabsorption: vit ADEK, Zn, Ca, Mg
Exocrine Faecal elastase C13 breath test
Endocrine Glucose, HbA1C
Weight height BMI AMC, TSF HGS BIA REE
Vit D, PTH GGT ALP DXA
Diet history and diet plan
Smoking alcohol physical activity compliance others

Figure 3 Nutritional assessment in patients with chronic pancreatitis. AMC: Arm muscle circumference; TSF: Triceps skin fold; HGS: Hand grip strength; BIA: Bioimpedance; REE: Resting energy expenditure; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; PTH: Parathyroid hormone; DXA: Dexa scan.

ever undetermined and in our cohort of ambulatory patients with CP we did not find any correlation between micronutrient deficiencies and handgrip strength as measured by bioimpedance (unpublished data). On the other hand we recently demonstrated that the fibrotic changes as well as atrophy and ductal-related parameters were associated with exocrine insufficiency such as reflected in vitamin D deficiency, and future studies should explore this in further details[28].

NUTRITIONAL ASSESSMENT

Assessment of nutrition status should include a multidisciplinary approach[14], including assessment of clinical symptoms, exocrine and endocrine pancreatic function, body composition, bone health, dietary evaluation and lifestyle as illustrated in Figure 3.

Clinical symptoms should include routine medical history and psychical examination with special emphasis on nutrition related symptoms and risk factors (nausea, anorexia, pain, alcohol, smoking). Micronutrient status should be measured 1-2 times a year including malabsorption of fat-soluble vitamins, minerals and trace elements. Function of pancreas concerning the exocrine- and endocrine part will not be discussed in this article.

Because both weight and BMI do not take body composition into account and may be misinterpreted as a result of fluid changes including ascites and edema, further investigation and assessment may be made with anthropometrics and bioimpedance measurements, both for baseline and follow-up (*i.e.*, every 3-6 mo), because even patients with a normal BMI may have a decreased muscle mass that further might decrease function and lead to a higher morbidity, *i.e.*, higher incidence of postoperative complications after surgery for CP[16].

Anthropometric measurements as mid-arm circumference estimating lean body mass and triceps skinfold estimating subcutaneous fat stores can be compared with age- and gender-specific centiles and are useful especially in patients with edema or ascites and as a long-term follow-up for nutritional status[14].

Bioimpedance is easy, non-invasive, and relatively inexpensive, and can be performed in almost any subject because it is portable. ESPEN guidelines[29-31], report results for fat-free mass body fat, body cell mass total body water, extracellular water and intracellular water from various studies in healthy and ill subjects. The data suggests that bioimpedance works well in healthy subjects and in patients with stable water and electrolytes balance with a validated bioimpedance equation that is appropriate with regard to age, sex and race. Clinical use of bioimpedance in subjects at extremes of BMI ranges or with abnormal hydration cannot be recommended for routine assessment of patients until further validation has proven for bioimpedance algorithm to be accurate in such conditions. Multi-frequency- and segmental-bioimpedance may have advantages over single-frequency bioimpedance in these conditions, but further validation is necessary. Longitudinal follow-up of body composition by bioimpedance is possible in subjects with BMI 16-34 kg/m^2 without abnormal hydration, but must be interpreted with caution.

Since muscle function correlates closely with whole body protein, body cell mass, anthropometrically measured arm muscle mass, and even with BMI, loss of weight or muscle mass invariably results in decreased muscle strength which is reflected in deteriorating function tests as well as in prominently altered muscle morphology[32]. Reduced muscle strength is in turn associated with loss of physical functionality and with negative impact on recovery of health after illness or surgery, which partly explains the high predictive power of muscle function tests[2]. Hence, various studies have shown a close correlation between muscle strength and outcome in acute and chronic disease[32-34]. Just as measuring body composition offers a qualitative aspect of nutritional status, muscle function represents a dynamic indicator of muscle mass. Measurement of muscle function as indicator of functional as well as nutritional status has therefore gained considerable attention in the past years. Although hand grip strength correlates well with other muscle function tests such as knee extension strength or peak expiratory flow, it cannot be used as surrogate for muscle function of lower extremities when evaluating physical performance. Short term effects of nutritional therapy as *e.g.*, refeeding of acute malnutrition are seen earlier by muscle function than by changes in body composition. Long-term nutritional therapy should result in both changes of body composition and muscle function, which should be paralleled by improvements of physical status[31,35].

Reference values for age- and gender-specific percentiles exist[36].

Resting energy expenditure (REE): Institution of appropriate nutritional therapy necessitates accurate determination of energy requirements. Data on measured REE in CP are very limited, but has shown, that weight loss is accompanied by hypermetabolism, and that between 30% and 50% of patients with CP have increased REE[20]. It may help us predict the energy level necessary to promote weight restoration and optimize nutritional rehabilitation preventing severe medical complications such as the refeeding syndrome[37]. Unfortunately, this technology is not available in the majority of the hospitals, because it requires skilled technicians and sophisticated methodologies that are costly and difficult to apply in standard clinical settings.

Predictive formulas of REE may be used as an alternative to indirect calorimetric that may be utilized by clinicians. The most cited and used predictive formula is the Harris-Benedict equation, which includes age, stature, and body weight to estimate REE[38]. However, studies are needed to validate predictive formulas *vs* measured resting energy expenditure by indirect calorimetric.

Bone health: Despite the reported high incidence of osteopathy in CP, no disease specific guideline exits for CP. However, extrapolating from guidelines for comparable malabsorptive diseases, calcium and vitamin D supplementation as well as regular monitoring of bone health should be an integral part of the nutrition management of CP, hence biochemistry and DXA scan should be performed regularly (*i.e.*, once a year).

Diet: A detailed assessment of current and habitual dietary intakes should be made by a dietician for all patients at nutritional risk as indicated by the NRS 2002 screening tool. Nutrient intake may be measured by a 24-h recall interview or a diet history, and analyzed using specialized software providing detailed information about energy- and protein intake as well as fat and micronutrients intake. For evaluating specific food items a food frequency questionnaire can be used[39]. A specific diet plan should be made and follow-up visits evaluating intake compared to recommended energy- and protein intake should be employed according to clinical monitoring practice. It has been shown, that both dietary counseling and oral supplements for CP patients at nutritional risk can improve weight, BMI and decrease fecal fat excretion[40].

Lifestyle: To minimize nutritional risk factors in patients with CP, an effort should be made to investigate and eliminate possible individual barriers such as smoking and alcohol abuse. Furthermore, to assure patient compliance regarding medical treatment (*i.e.*, treatment with enzymes) and to evaluate pain treatment (discussed in another article). Physical activity should be encouraged alongside nutritional therapy for optimal result[32-34].

NUTRITIONAL MANAGEMENT

Nutritional treatment in chronic pancreatitis

Maldigestion of macronutrients is the major cause of progressive nutritional and metabolic impairment in patients with CP. Nutritional interventions depend on the degree of maldigestion and the nutritional status.

The main goals for nutritional interventions are to ensure sufficient macro- and micronutrients intake, to decrease maldigestion, malabsorption and other risk factors in order to prevent or treat malnutrition.

The treatment of exocrine deficiency begins with dietary recommendations and pancreatic enzyme supplementation. About 80% of patients can be managed by a combination of analgesics, dietary recommendations and pancreatic enzyme supplements, while 10%-15% need oral nutritional supplements, 5% need enteral tube feeding and around 1% require parenteral nutrition[21,41,42].

Diet recommendations

Dietary recommendations begin with total abstinence from alcohol. In addition, an adequate number of calories should be taken. Estimation of REE [or measurement in patients with a low BMI (< 20 kg/m^2)] is essential in all patients to calculate the adequate caloric intake because of risk of increased resting energy expenditure. Frequent small meals (4-8 times a day) should be given. The carbohydrate intake might be limited when an overt diabetes mellitus is present (described in more detail in other article).

A protein diet of 1.0-1.5 g/kg body weight/d is generally sufficient and well tolerated. Usually, if 30%-40% of the calories are given as fat this is well tolerated, especially when the foods are rich in vegetable fats.

If weight gain is insufficient and/or steatorrhea persists, medium chain triglycerides (MCT) can be tried to increase fat absorption. MCT are absorbed directly across the small bowel into the portal vein, even in the absence of lipase, co-lipase and bile salts. However, MCTs have low energy density and unpalatable taste, and a maximum of about 50 g/d might be given. Higher doses may be ketogenic and are associated with side effects such as cramps, nausea and diarrhea. Fat soluble vitamins (A, D, E and K), vitamin B12 and other micronutrients should be supplemented if serum levels indicate deficiencies.

In general, a low fibre diet is recommended, because fibre may absorb enzymes and delay the absorption of nutrients. An adequate quantity of exogenous pancreatic enzymes is necessary to correct protein and lipid maldigestion[1,43-45]. In 10%-15% of patients oral supplements can help to attenuate weight loss and delay the use of enteral tube feeding[42,46].

The best clinical follow-up parameters for monitoring therapeutic success of dietary counseling are improvement of the patient's general condition and weight gain.

Enteral nutrition

The cause of inadequate caloric intake in CP can be anatomical (due to pyloro-duodenal-stenosis or cyst compression) or inflammatory with acute complications (new attack of acute pancreatitis or development of fistulas). Patients suffering from serious insufficient caloric intake may benefit by oral supplements or enteral nutrition. To test if enteral nutrition is tolerated and increases nutritional status it is recommended to give the nutrition via a naso-jejunal tube. However, for long-term therapy feeding (exceeding 2-3 wk) a percutaneous endoscopic gastrostomy with a jejunal tube extension is more convenient. Continuous overnight delivery of the nutrients is suitable and entails more easily the patient's nutritional goal. From a theoretical point of view a semi-elemental diet can be recommended, but there are no studies showing improvement in the nutritional status compared to regular enteral nutritional formulas.

Owing to the fact that CP patients are frequently undernourished, nutritional support before pancreatic surgery may be beneficial. Data from patients undergoing general abdominal surgery have provided evidence that preoperative enteral or oral nutritional support improves outcome compared to undernourished patients by reducing postoperative morbidity and the length of hospital stay[47]. Thus, it should be emphasized that nutritional therapy should go alongside surgery, and that surgery for pain or any obstruction in the GI-channel should be a primary indication.

The potential to modulate the activity of the immune system by interventions with specific nutrients is termed immunonutrition. This concept is normally applied to any situation where nutritional formulas are supplemented with specific nutrients such as arginine, glutamine, omega-3 fatty acids, nucleotides and others. Meta-analysis of randomized controlled trials giving a combination of arginine and omega-3 fatty acids at home for 5-7 d before surgery reduced both postoperative infection rate and length of hospital stay in patients who underwent elective major GI surgery[48,49]. Specific trials in patients with chronic pancreatitis are not available. A recent RCT giving supplemental immunonutrition preoperatively in well-nourished and mal-nourished gastrointestinal surgery patients showed, that LOS and costs were reduced especially in mal-nourished patients[50].

Another recent study showed that enteral immunonutrition given post-operatively *vs* a standard enteral nutrition showed a reduction of infectious complications, anastomotic leak rate and LOS as well as an improved immunologic outcome[51].

We therefore highly recommend a week with preoperative immunonutrition to patients going to elective surgery for chronic pancreatitis. Early postoperative enteral nutrition is feasible and may additionally improve outcome after surgery.

Parenteral nutrition

Parenteral nutrition is infrequently used in patients with chronic pancreatitis. Enteral nutrition preserves immune function and mucosal architecture and decreases the possibility for hyperglycemia while parenteral nutrition also increases the risk of catheter infections and sepsis complications. Parenteral nutrition is therefore only indicated when it is impossible to use enteral nutrition[52]. This means if the patients do not reach their requirements because gastric emptying is blocked, the patient needs gastric decompression, it is impossible to introduce a tube into the jejunum, or a complicated fistula is present. Parenteral nutrition is mainly performed over a short term period, *e.g.*, in apparent severe malnutrition prior to pancreatic surgery if enteral feeding is incomplete and may thus be used as a supplement to fulfill their requirements. There are no reported studies of patients with chronic pancreatic insufficiency who have been treated with parenteral nutrition for a long period.

Future perspectives

Future studies evaluating a systematic approach for assessment and treatment of CP patients at nutritional risk should be made to further elucidate the nutritional course of these patients. Special emphasis should be on body composition, absorption of nutrients and metabolism, as well as measuring micronutrient deficiencies. Furthermore, nutritional interventions should be tested in randomized controlled trials with relevant clinical outcomes, *i.e.*, morbidity, quality of life, physical function and mortality.

REFERENCES

1 **Sikkens EC**, Cahen DL, van Eijck C, Kuipers EJ, Bruno MJ. Patients with exocrine insufficiency due to chronic pancreatitis are undertreated: a Dutch national survey. *Pancreatology* 2012; **12**: 71-73 [PMID: 22487479 DOI: 10.1016/j.pan.2011.12.010]

2 **Armbrecht U**. [Chronic pancreatitis: weight loss and poor physical performance - experience from a specialized rehabilitation centre]. *Rehabilitation (Stuttg)* 2001; **40**: 332-336 [PMID: 11742423 DOI: 10.1055/s-2001-18966]

3 **Sorensen J**, Kondrup J, Prokopowicz J, Schiesser M, Krähenbühl L, Meier R, Liberda M. EuroOOPS: an international, multicentre study to implement nutritional risk screening and evaluate clinical outcome. *Clin Nutr* 2008; **27**: 340-349 [PMID: 18504063 DOI: 10.1016/j.clnu.2008.03.012]

4 **Rasmussen HH**, Kondrup J, Staun M, Ladefoged K, Kristensen H, Wengler A. Prevalence of patients at nutritional risk in Danish hospitals. *Clin Nutr* 2004; **23**: 1009-1015 [PMID: 15380890 DOI: 10.1016/j.clnu.2004.01.001]

5 **Johansen N**, Kondrup J, Plum LM, Bak L, Nørregaard P, Bunch E, Baernthsen H, Andersen JR, Larsen IH, Martinsen A. Effect of nutritional support on clinical outcome in patients at nutritional risk. *Clin Nutr* 2004; **23**: 539-550 [PMID: 15297090 DOI: 10.1016/j.clnu.2003.10.008]

6 **Kondrup J**, Allison SP, Elia M, Vellas B, Plauth M. ESPEN guidelines for nutrition screening 2002. *Clin Nutr* 2003; **22**: 415-421 [PMID: 12880610]

7 **Jie B**, Jiang ZM, Nolan MT, Zhu SN, Yu K, Kondrup J. Impact of preoperative nutritional support on clinical outcome in abdominal surgical patients at nutritional risk. *Nutrition* 2012; **28**: 1022-1027 [PMID: 22673593 DOI: 10.1016/j.nut.2012.01.017]

8 **Weekes CE**, Spiro A, Baldwin C, Whelan K, Thomas JE, Parkin D, Emery PW. A review of the evidence for the impact of improving nutritional care on nutritional and clinical outcomes and cost. *J Hum Nutr Diet* 2009; **22**: 324-335 [PMID: 19624401 DOI: 10.1111/j.1365-277X.2009.00971.x]

9 **Kondrup J**, Rasmussen HH, Hamberg O, Stanga Z. Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. *Clin Nutr* 2003; **22**: 321-336 [PMID: 12765673]

10 **Soeters PB**, Reijven PL, van Bokhorst-de van der Schueren MA, Schols JM, Halfens RJ, Meijers JM, van Gemert WG. A rational approach to nutritional assessment. *Clin Nutr* 2008; **27**: 706-716 [PMID: 18783855 DOI: 10.1016/j.clnu.2008.07.009]

11 **Jensen GL**, Mirtallo J, Compher C, Dhaliwal R, Forbes A, Grijalba RF, Hardy G, Kondrup J, Labadarios D, Nyulasi I, Castillo Pineda JC, Waitzberg D. Adult starvation and disease-related malnutrition: a proposal for etiology-based diagnosis in the clinical practice setting from the International Consensus Guideline Committee. *JPEN J Parenter Enteral Nutr* 2010; **34**: 156-159 [PMID: 20375423 DOI: 10.1177/0148607110361910]

12 **Jensen GL**. Inflammation: an expanding universe. *Nutr Clin Pract* 2008; **23**: 1-2 [PMID: 18203959]

13 **Botta D**, Gauthier AP. [Indications of enteral nutrition in pancreatic disorders]. *Ann Gastroenterol Hepatol (Paris)* 1988; **24**: 335-338 [PMID: 3145704]

14 **Duggan S**, O'Sullivan M, Feehan S, Ridgway P, Conlon K. Nutrition treatment of deficiency and malnutrition in chronic pancreatitis: a review. *Nutr Clin Pract* 2010; **25**: 362-370 [PMID: 20702842 DOI: 10.1177/0884533610373772]

15 **Affronti J**. Chronic pancreatitis and exocrine insufficiency. *Prim Care* 2011; **38**: 515-37; ix [PMID: 21872095 DOI: 10.1016/j.pop.2011.05.007]

16 **Riediger H**, Adam U, Fischer E, Keck T, Pfeffer F, Hopt UT, Makowiec F. Long-term outcome after resection for chronic pancreatitis in 224 patients. *J Gastrointest Surg* 2007; **11**: 949-59; discussion 959-60 [PMID: 17534689 DOI: 10.1007/s11605-007-0155-6]

17 **Haaber AB**, Rosenfalck AM, Hansen B, Hilsted J, Larsen S. Bone mineral metabolism, bone mineral density, and body composition in patients with chronic pancreatitis and pancreatic exocrine insufficiency. *Int J Pancreatol* 2000; **27**: 21-27 [PMID: 10811020]

18 **Fitzsimmons D**, Kahl S, Butturini G, van Wyk M, Bornman P, Bassi C, Malfertheiner P, George SL, Johnson CD. Symptoms and quality of life in chronic pancreatitis assessed by structured interview and the EORTC QLQ-C30 and QLQ-PAN26. *Am J Gastroenterol* 2005; **100**: 918-926 [PMID: 15784041 DOI: 10.1111/j.1572-0241.2005.40859.x]

19 **Holst M**, Schou-Olesen S, Kohler M, Drewes AM, Rasmussen HH. Nutritional assessment in ambulatory patients with chronic pancreatitis. *Clin Nutr Suppl* 2013; **32** Suppl 1: 47

20 **Hébuterne X**, Hastier P, Péroux JL, Zeboudj N, Delmont JP, Rampal P. Resting energy expenditure in patients with alcoholic chronic pancreatitis. *Dig Dis Sci* 1996; **41**: 533-539 [PMID: 8617130]

21 **Meier RF**, Beglinger C. Nutrition in pancreatic diseases. *Best Pract Res Clin Gastroenterol* 2006; **20**: 507-529 [PMID: 16782526 DOI: 10.1016/j.bpg.2006.01.004]

22 **Dujsikova H**, Dite P, Tomandl J, Sevcikova A, Precechtelova M. Occurrence of metabolic osteopathy in patients with chronic pancreatitis. *Pancreatology* 2008; **8**: 583-586 [PMID: 18824882 DOI: 10.1159/000159845]

23 **Kalvaria I**, Labadarios D, Shephard GS, Visser L, Marks IN. Biochemical vitamin E deficiency in chronic pancreatitis. *Int J Pancreatol* 1986; **1**: 119-128 [PMID: 3693979 DOI: 10.1007/BF02788444]

24 **Nakamura T**, Tando Y. [Study on pancreatic steatorrhea in patients with chronic pancreatitis]. *Nihon Shokakibyo Gakkai Zasshi* 2000; **97**: 1347-1354 [PMID: 11215184]

25 **Bang UC**, Benfield T, Bendtsen F, Hyldstrup L, Beck Jensen JE. The Risk of Fractures Among Patients With Cirrhosis or Chronic Pancreatitis. *Clin Gastroenterol Hepatol* 2013; Epub ahead of print [PMID: 23644391 DOI: 10.1016/j.cgh.2013.04.031]

26 **Glasbrenner B**, Malfertheiner P, Büchler M, Kuhn K, Ditschuneit H. Vitamin B12 and folic acid deficiency in chronic pancreatitis: a relevant disorder? *Klin Wochenschr* 1991; **69**: 168-172 [PMID: 2041378]

27 **Quilliot D**, Walters E, Bonte JP, Fruchart JC, Duriez P, Ziegler O. Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis. *Am J Clin Nutr* 2005; **81**: 1117-1125 [PMID: 15883437]

28 **Frokjaer J**, Olesen SS, Drewes AM. Fibrosis, atrophy and ductal pathology in chronic pancreatitis are associated with pancreatic function but independent of symptoms. *Pancreas* 2013; In press

29 **Kyle UG**, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, Heitmann BL, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AM, Pichard C. Bioelectrical impedance analysis--part I: review of principles and methods. *Clin Nutr* 2004; **23**: 1226-1243 [PMID: 15380917 DOI: 10.1016/j.clnu.2004.06.004]

30 **Kyle UG**, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gómez J, Lilienthal Heitmann B, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, M W J Schols A, Pichard C. Bioelectrical impedance analysis-part II: utilization in clinical practice. *Clin Nutr* 2004; **23**: 1430-1453 [PMID: 15556267 DOI: 10.1016/j.clnu.2004.09.012]

31 **Norman K**, Pirlich M, Sorensen J, Christensen P, Kemps M, Schütz T, Lochs H, Kondrup J. Bioimpedance vector analysis as a measure of muscle function. *Clin Nutr* 2009; **28**: 78-82 [PMID: 19064305 DOI: 10.1016/j.clnu.2008.11.001]

32 **Tieland M**, Dirks ML, van der Zwaluw N, Verdijk LB, van de Rest O, de Groot LC, van Loon LJ. Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* 2012; **13**: 713-719 [PMID: 22770932 DOI: 10.1016/j.jamda.2012.05.020]

33 **Candow DG**, Forbes SC, Little JP, Cornish SM, Pinkoski C, Chilibeck PD. Effect of nutritional interventions and resistance exercise on aging muscle mass and strength. *Biogerontology* 2012; **13**: 345-358 [PMID: 22684187 DOI: 10.1007/s10522-012-9385-4]

34 **Geirsdottir OG**, Arnarson A, Briem K, Ramel A, Jonsson PV, Thorsdottir I. Effect of 12-week resistance exercise program on body composition, muscle strength, physical function, and glucose metabolism in healthy, insulin-resistant, and diabetic elderly Icelanders. *J Gerontol A Biol Sci Med Sci* 2012; **67**: 1259-1265 [PMID: 22496538 DOI: 10.1093/gerona/gls096]

35 **Norman K**, Stobäus N, Gonzalez MC, Schulzke JD, Pirlich M. Hand grip strength: outcome predictor and marker of nu-

tritional status. *Clin Nutr* 2011; **30**: 135-142 [PMID: 21035927 DOI: 10.1016/j.clnu.2010.09.010]

36 **Schlüssel MM**, dos Anjos LA, de Vasconcellos MT, Kac G. Reference values of handgrip dynamometry of healthy adults: a population-based study. *Clin Nutr* 2008; **27**: 601-607 [PMID: 18547686 DOI: 10.1016/j.clnu.2008.04.004]

37 **Słodkowski M**, Rubinsztajn R, Cebulski W, Krasnodebski IW. [A case report of severe hypophosphatemia in the course of refeeding syndrome]. *Pol Merkur Lekarski* 2004; **17**: 638-639 [PMID: 15771140]

38 **Harris JA**, Benedict FG. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci USA* 1918; **4**: 370-373 [PMID: 16576330]

39 **Streppel MT**, de Vries JH, Meijboom S, Beekman M, de Craen AJ, Slagboom PE, Feskens EJ. Relative validity of the food frequency questionnaire used to assess dietary intake in the Leiden Longevity Study. *Nutr J* 2013; **12**: 75 [PMID: 23758629 DOI: 10.1186/1475-2891-12-75]

40 **Singh S**, Midha S, Singh N, Joshi YK, Garg PK. Dietary counseling versus dietary supplements for malnutrition in chronic pancreatitis: a randomized controlled trial. *Clin Gastroenterol Hepatol* 2008; **6**: 353-359 [PMID: 18328440 DOI: 10.1016/j.cgh.2007.12.040]

41 **Meier R**, Ockenga J, Pertkiewicz M, Pap A, Milinic N, Macfie J, Löser C, Keim V. ESPEN Guidelines on Enteral Nutrition: Pancreas. *Clin Nutr* 2006; **25**: 275-284 [PMID: 16678943 DOI: 10.1016/j.clnu.2006.01.019]

42 **Plauth M**, Cabré E, Campillo B, Kondrup J, Marchesini G, Schütz T, Shenkin A, Wendon J. ESPEN Guidelines on Parenteral Nutrition: hepatology. *Clin Nutr* 2009; **28**: 436-444 [PMID: 19520466 DOI: 10.1016/j.clnu.2009.04.019]

43 **Nakajima K**, Oshida H, Muneyuki T, Kakei M. Pancrelipase: an evidence-based review of its use for treating pancreatic exocrine insufficiency. *Core Evid* 2012; **7**: 77-91 [PMID: 22936895 DOI: 10.2147/CE.S26705]

44 **Domínguez-Muñoz JE**. Chronic pancreatitis and persistent steatorrhea: what is the correct dose of enzymes? *Clin Gastroenterol Hepatol* 2011; **9**: 541-546 [PMID: 21377551 DOI: 10.1016/j.cgh.2011.02.027]

45 **Domínguez-Muñoz JE**, Iglesias-García J. Oral pancreatic enzyme substitution therapy in chronic pancreatitis: is clinical response an appropriate marker for evaluation of therapeutic efficacy? *JOP* 2010; **11**: 158-162 [PMID: 20208327]

46 **Lochs H**, Dejong C, Hammarqvist F, Hebuterne X, Leon-Sanz M, Schütz T, van Gemert W, van Gossum A, Valentini L, Lübke H, Bischoff S, Engelmann N, Thul P. ESPEN Guidelines on Enteral Nutrition: Gastroenterology. *Clin Nutr* 2006; **25**: 260-274 [PMID: 16698129 DOI: 10.1016/j.clnu.2006.01.007]

47 **Weimann A**, Braga M, Harsanyi L, Laviano A, Ljungqvist O, Soeters P, Jauch KW, Kemen M, Hiesmayr JM, Horbach T, Kuse ER, Vestweber KH. ESPEN Guidelines on Enteral Nutrition: Surgery including organ transplantation. *Clin Nutr* 2006; **25**: 224-244 [PMID: 16698152 DOI: 10.1016/j.clnu.2006.01.015]

48 **Cerantola Y**, Hübner M, Grass F, Demartines N, Schäfer M. Immunonutrition in gastrointestinal surgery. *Br J Surg* 2011; **98**: 37-48 [PMID: 20931620 DOI: 10.1002/bjs.7273]

49 **Marik PE**, Zaloga GP. Immunonutrition in high-risk surgical patients: a systematic review and analysis of the literature. *JPEN J Parenter Enteral Nutr* 2010; **34**: 378-386 [PMID: 20631383 DOI: 10.1177/0148607110362692]

50 **Barker LA**, Gray C, Wilson L, Thomson BN, Shedda S, Crowe TC. Preoperative immunonutrition and its effect on postoperative outcomes in well-nourished and malnourished gastrointestinal surgery patients: a randomised controlled trial. *Eur J Clin Nutr* 2013; **67**: 802-807 [PMID: 23801093 DOI: 10.1038/ejcn.2013.117]

51 **Marano L**, Porfidia R, Pezzella M, Grassia M, Petrillo M, Esposito G, Braccio B, Gallo P, Boccardi V, Cosenza A, Izzo G, Di Martino N. Clinical and immunological impact of early postoperative enteral immunonutrition after total gastrectomy in gastric cancer patients: a prospective randomized study. *Ann Surg Oncol* 2013; **20**: 3912-3918 [PMID: 23838912 DOI: 10.1245/s10434-013-3088-1]

52 **Seres DS**, Valcarcel M, Guillaume A. Advantages of enteral nutrition over parenteral nutrition. *Therap Adv Gastroenterol* 2013; **6**: 157-167 [PMID: 23503324 DOI: 10.1177/1756283X12467564]

P- Reviewers: Keck T, Kochhar R **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Zhang DN

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doi:10.3748/wjg.v19.i42.7276

World J Gastroenterol 2013 November 14; 19(42): 7276-7281
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Diagnosis and treatment of diabetes mellitus in chronic pancreatitis

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Received: June 10, 2013 **Revised:** August 13, 2013
Accepted: September 4, 2013
Published online: November 14, 2013

Abstract

Diabetes secondary to pancreatic diseases is commonly referred to as pancreatogenic diabetes or type 3c diabetes mellitus. It is a clinically relevant condition with a prevalence of 5%-10% among all diabetic subjects in Western populations. In nearly 80% of all type 3c diabetes mellitus cases, chronic pancreatitis seems to be the underlying disease. The prevalence and clinical importance of diabetes secondary to chronic pancreatitis has certainly been underestimated and underappreciated so far. In contrast to the management of type 1 or type 2 diabetes mellitus, the endocrinopathy in type 3c is very complex. The course of the disease is complicated by additional present comorbidities such as maldigestion and concomitant qualitative malnutrition. General awareness that patients with known and/or clinically overt chronic pancreatitis will develop type 3c diabetes mellitus (up to 90% of all cases) is rather good. However, in a patient first presenting with diabetes mellitus, chronic pancreatitis as a potential causative condition is seldom considered. Thus many patients are misdiagnosed. The failure to correctly diagnose type 3 diabetes mellitus leads to a failure to implement an appropriate medical therapy. In patients with type 3c diabetes mellitus treating exocrine pancreatic insufficiency, preventing or treating a lack of fat-soluble vitamins (especially vitamin D) and restoring impaired fat hydrolysis and incretin secretion are key-features of medical therapy.

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Key words: Diabetes mellitus; Chronic pancreatitis; Type 3c diabetes; Pancreatogenic diabetes; Pancreatitis

Core tip: Type 3c diabetes mellitus is more common than generally thought. Its prevalence is supposed to be among 5%-10% among all diabetics. Most patients with type 3c diabetes mellitus suffer from chronic pancreatitis as the underlying disease. Misclassification of these patients is very common, yet identification of these patients is very important due to some special diagnostic and therapeutic considerations in this subset of patients. Among these are *e.g.*, restoring proper fat assimilation, preventing fat-soluble vitamin deficiency and early identification of pancreatic cancer patients. Specific diagnostic criteria for type 3c diabetes mellitus are proposed within this review.

Ewald N, Hardt PD. Diagnosis and treatment of diabetes mellitus in chronic pancreatitis. *World J Gastroenterol* 2013; 19(42): 7276-7281 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7276.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7276

INTRODUCTION

Chronic pancreatitis is a disease characterized by pancreatic inflammatory and fibrotic injury resulting in irreversible parenchymal damage. Progressive nutrient maldigestion and disturbance of the timing and the interactions

Table 1 Current classification of diabetes mellitus

I	Type 1 Diabetes Mellitus (β-cell destruction, usually leading to absolute insulin deficiency)
	A: Immune mediated
	B: Idiopathic
II	Type 2 Diabetes Mellitus (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III	Other Specific Types Of Diabetes Mellitus
	A: Genetic defects of β-cell function
	B: Genetic defects in insulin action
	C: Diseases of the exocrine pancreas
	1: Pancreatitis
	2: Trauma/pancreatectomy
	3: Neoplasia
	4: Cystic fibrosis
	5: Hemochromatosis
	6: Fibrocalculous pancreatopathy
	7: Others
	D: Endocrinopathies
	E: Drug- or chemical-induced
	F: Infections
	G: Uncommon forms of immune-mediated diabetes
	H: Other genetic syndromes sometimes associated with diabetes
IV	Gestational Diabetes Mellitus

Source: Ref. [4,5], with permission.

between nutrient digestion and absorption is observed and may lead to severe metabolic derangements. Glucose intolerance and diabetes mellitus are observed quite frequently in the course of the disease[1,2].

Development of diabetes mellitus in chronic pancreatitis mainly occurs due to the destruction of islet cells by pancreatic inflammation. Additionally, nutrient maldigestion leads to an impaired incretin secretion and therefore to a diminished insulin release of the remaining beta-cells[3]. In contrast to the autoimmune mediated destruction of the beta-cells in type 1 diabetes mellitus, glucagon secreting alpha-cells and pancreatic polypeptide secreting pancreatic polypeptide-cells are also subject to destruction in chronic pancreatitis leading to a complex deranged metabolic situation.

Diabetes mellitus secondary to pancreatic diseases (such as chronic pancreatitis) is classified as pancreatogenic diabetes or type 3c diabetes mellitus according to the current classification of diabetes mellitus (Table 1)[4,5]. Whereas the awareness of type 1 and type 2 diabetes mellitus is rather good, type 3c diabetes mellitus, however, is a condition rarely considered in everyday practice. Yet, recent data on type 3c diabetes mellitus show that it might be more common than generally thought. Studies also suggest that this important condition might be consistently under- and misdiagnosed[6,7].

Due to the complex pathophysiology of type 3c diabetes mellitus it bears clinical and laboratory features which are very distinct from both type 1 and type 2 diabetes mellitus. This review focuses on diagnosis and treatment of diabetes mellitus secondary to chronic pancreatitis.

PREVALENCE OF DIABETES MELLITUS SECONDARY TO PANCREATIC DISEASES (TYPE 3C)

In contrast to type 1 and type 2 diabetes mellitus, detailed data on the prevalence of type 3c diabetes mellitus hardly exist. Some older studies estimate a rather low prevalence of about 0.5%-1.15% among all cases of diabetes mellitus in North America[8,9]. Other studies from, *e.g.*, Southeast Asia where tropical or fibrocalcific pancreatitis is endemic, report a higher prevalence of approximately 15%-20% of all diabetes mellitus cases[10,11].

A recent review of the currently available studies on this topic proposes a prevalence of 5%-10% for type 3c diabetes mellitus among all diabetes mellitus cases in Western populations[12]. Data are mainly based on a large retrospective study of 1868 patients at a German University Hospital, where type 3c diabetes mellitus accounted for 9.2% of all diabetics[7]. This emphasizes that previous older estimates of the prevalence of type 3c diabetes mellitus must be inaccurately low. In 78.5% of all patients with type 3c diabetes mellitus, chronic pancreatitis was identified as the underlying diseases, therefore resembling the most important causative condition[7].

The previous underestimation of the prevalence of type 3c diabetes mellitus might partly be due to the fact that investigation of the pancreas has meanwhile been facilitated by new diagnostic procedures. Nowadays it has become much easier to detect exocrine pancreatic pathology as imaging methods of the pancreas have clearly improved and noninvasive screening methods to quantify exocrine pancreatic insufficiency are easily available.

If chronic pancreatitis accounts for nearly 80% of all type 3c diabetes mellitus cases, and if the prevalence of type 3c diabetes mellitus is expected to be approximately 5%-10% of all diabetes mellitus cases, the true prevalence of (subclinical) chronic pancreatitis in the general population seems to be far underestimated. This might especially hold true since chronic pancreatitis has previously been considered a disease of alcoholism until the discovery that it is a multifactorial disease with an impact of complex genetic genotypes, smoking, special anatomic conditions, toxic agents and autoimmunity, also[13]. Up to date quite a few autopsy studies[14-16], endoscopic ultrasound studies[17] and exocrine pancreatic function studies[18] report a high frequency of exocrine pancreatic injury suggestive of chronic pancreatitis in the general population. This further supports the view of an underestimation of chronic (subclinical) pancreatitis in the general population.

DIAGNOSIS OF DIABETES MELLITUS IN CHRONIC PANCREATITIS

As stated above glucose intolerance and diabetes mellitus are common in chronic pancreatitis. Diagnosing diabetes

Table 2 Proposed diagnostic criteria for type 3c diabetes mellitus

Major criteria (must be present)
Presence of exocrine pancreatic insufficiency (monoclonal fecal elastase-1 test or direct function tests)
Pathological pancreatic imaging (endoscopic ultrasound, MRI, CT)
Absence of type 1 diabetes mellitus associated autoimmune markers
Minor criteria
Absent pancreatic polypeptide secretion
Impaired incretin secretion (*e.g.*, GLP-1)
No excessive insulin resistance (*e.g.*, HOMA-IR)
Impaired beta cell function (*e.g.*, HOMA-B, C-Peptide/glucose-ratio)
Low serum levels of lipid soluble vitamins (A, D, E and K)

MRI: Magnetic resonance imaging; CT: Computed tomography; GLP-1: Glucagon-like peptide-1; HOMA-IR: Homeostasis model assessment of insulin resistance; HOMA-B: Homeostasis model assessment of beta-cell.

mellitus in a patient with known chronic pancreatitis may not be that difficult. Yet, the correct classification of type 3c diabetes mellitus is often missed and patients are commonly misclassified. In a German study only about half of the cases of type 3c diabetes mellitus were classified correctly. Type 3c diabetes mellitus patients were mostly misclassified as type 2 diabetes[7]. This might be due to the very poor awareness of this diabetes type.

However, another thing appears even more difficult: do not forget to take into account that a patient first presenting with diabetes mellitus might have a type 3c diabetes mellitus. In any case of a new diabetes mellitus manifestation we should truly use the classification criteria defined by the European Association on the Study of Diabetes (EASD) and the American Diabetes Association (ADA)[4,5] and check for type 3c diabetes mellitus. At least if a patient does not fit into the common presentation and complains about gastrointestinal symptoms the physician should be aware of the existence of type 3c and initiate further diagnostics.

Screening for type 3c diabetes mellitus in chronic pancreatitis

Any patient with chronic pancreatitis should of course be monitored for the development of type 3c diabetes mellitus. The prevalence of diabetes mellitus among patients with an established diagnosis of chronic pancreatitis is reported to be up to 70% (in chronic calcific pancreatitis even up to 90%)[1,2]. Patients with long-standing duration of the disease, prior partial pancreatectomy, and early onset of calcific disease seem to be at higher risk for developing type 3c diabetes mellitus. There is a clear increase in the prevalence with the duration of chronic pancreatitis[19,20].

The initial evaluation of patients with chronic pancreatitis should include fasting glucose and HbA_{1c}. These tests should be repeated at least annually. Impairment in either one requires further evaluation. If testing suggests an impaired glucose tolerance, further evaluation by a 75 g oral glucose tolerance test is recommended[21]. A concomitant analysis of insulin and/or C-peptide levels may be helpful in distinguishing between type 2 and type 3c diabetes mellitus[22].

Distinguishing type 3c diabetes from other types

It is not always easy to diagnose and classify a patient with type 3c diabetes mellitus correctly. Long-standing type 1 and type 2 diabetes mellitus patients are associated with exocrine pancreatic failure[23] and patients with diabetes mellitus are at a higher risk for developing acute and/or chronic pancreatitis anyway[24,25]. Patients with previous episodes of pancreatitis may also develop type 1 or type 2 diabetes independently of their exocrine pancreatic disease. In order to classify patients with type 3c diabetes mellitus correctly, commonly accepted diagnosis criteria should be established.

In distinguishing between the different diabetes types the presence of islet cell antibodies is consistent with type 1 diabetes mellitus, and the presence of clinical or biochemical evidence of insulin resistance is associated with type 2 diabetes mellitus. Due to the lack of commonly accepted diagnostic criteria up to date, we propose the following criteria for diagnosing type 3c diabetes mellitus (Table 2).

The evaluation of pancreatic polypeptide response to insulin-induced hypoglycemia, secretin-infusion or a mixed nutrient ingestion might be of additional diagnostic interest as discussed elsewhere[21]. An absent pancreatic polypeptide response is able to distinguish between type 3c diabetes mellitus from early type 1 and may also distinguish type 3c from type 2, which is characterized by elevated pancreatic polypeptide levels[26-28]. Routinely testing of incretin secretion or pancreatic polypeptide response in everyday practice, however, does not seem feasible.

TREATMENT OF DIABETES MELLITUS SECONDARY TO CHRONIC PANCREATITIS

Managing hyperglycemia

The derangement in glucose metabolism in type 3c diabetes mellitus ranges from a mild impairment to a severe form characterized by frequent episodes of hypoglycemia, commonly referred to as brittle diabetes[9]. In type 3c diabetes mellitus, blood glucose control may be unstable due to the loss of glucagon response to hypoglycemia, carbohydrate malabsorption and/or inconsistent eating patterns due to concomitant pain and/or nausea or chronic alcohol abuse. Thus it is generally reported that type 3c diabetes mellitus is difficult to control, although there are only very few studies in this field[29,30]. Astonishingly, all large clinical trials, including Diabetes Control and Complications Trial[31] and United Kingdom Prospective Diabetes Study [32] specifically excluded patients with type 3c diabetes mellitus.

Currently, there are no generally accepted guidelines regarding treatment pathways for type 3c diabetes mellitus. Yet, a first step was taken at Pancreas Fest 2012[21]. The pharmacological agents typically used for the treatment of type 3c diabetes mellitus are the same as for type 2 diabetes mellitus. The ADA and the EASD recommend metformin as the first-line oral therapy for type 2 diabetes mellitus[33]. Therefore many type 3c diabetes mellitus patients are initially treated with metformin as a drug of first choice. If hyperglycemia is rather mild and concomitant insulin resistance is additionally diagnosed or suspected, therapy with metformin may be a good choice in the absence of contraindications. However, metformin treatment might not be tolerated by a majority of patients since its main side effects include nausea, abdominal complaints, diarrhea and weight reduction. A patient with chronic pancreatitis will probably not tolerate these symptoms. Since metformin therapy proofs capable of reducing the risk of pancreatic cancer by as much as 70%, however, its anti-diabetic and anti-neoplastic effects may be beneficial in patients with type 3c diabetes mellitus due to chronic pancreatitis[34]. This holds especially true since chronic pancreatitis and diabetes mellitus are both well accepted risk factors for the development of pancreatic cancer[35-37].

Incretin based therapies [*e.g.,* glucagon-like peptide-1, (GLP-1-)analogues, dipeptidyl peptidase (DPP)-Ⅳ-inhibitors] also enhance insulin secretion. Yet, GLP-1-analogues as well as DPP-Ⅳ-inhibitors are both associated with a higher risk of pancreatitis and are reported to have a high frequency of prominent gastrointestinal side effects (*e.g.*, nausea, delayed gastric emptying, weight loss)[38]. Therefore their use should best be avoided at present time until their safety is confirmed. A better and probably safer way to positively influence the incretin system might be a proper supplementation with pancreatic enzymes in these patients as discussed below.

In early type 3c diabetes mellitus, oral therapy with insulin segretagogues (sulfonylurea and glinides) may also be considered, thiazolidines should be avoided due to prominent side effects (*e.g.*, bone fractures, fluid retention, congestive heart disease).

Chronic pancreatitis, however, must be seen as a progressive disorder and many patients will eventually require insulin therapy. Patients should then be treated using general insulin dosing guidelines as established for type 1 diabetes mellitus. In patients with severe malnutrition insulin therapy is commonly used as a therapy of first choice. This is due to the desired anabolic effects of insulin in this special subset of patients.

Insulin pump therapy may also be considered for patients who experience a brittle form of diabetes mellitus despite being sufficiently motivated.

As it is in the other diabetes types, initial treatment should include all efforts to correct lifestyle factors which contribute to hyperglycemia and the risk of pancreatic malignancy (*e.g.,* abstinence from alcohol and smoking cessation, weight loss in overweight subjects, physical exercise and dietary modifications).

Managing exocrine pancreatic insufficiency

Many patients with chronic pancreatitis manifest some degree of fat malabsorption, regardless of the presence of symptoms. In patients with type 3c diabetes mellitus exocrine pancreatic insufficiency is nearly ubiquitous present. Since clinically overt steatorrhea is usually not observed until over 90% of exocrine pancreatic function have vanished, exocrine pancreatic insufficiency and maldigestion might remain undetected. However, the relevant maldigestion, which is present in the majority of patients with chronic pancreatitis, may cause qualitative malnutrition. This is especially important concerning the absorption of fat-soluble vitamins (A, D, E and K).

Very recent studies show a vitamin D deficiency in > 90% of patients with chronic pancreatitis[39,40]. Additionally a significant correlation of exocrine pancreatic insufficiency and osteoporosis and/or alterations in bone metabolism can be observed[41,42].

Further considering the possible role of vitamin D deficiency in the pathogenesis of type 1 diabetes mellitus and the association of low vitamin D levels and poor glycemic control in observational studies[43,44], qualitative malnutrition of vitamin D in patients with type 3c diabetes mellitus seems of clinical importance. Measuring serum-25-hydroxyvitamin D levels and supplementing deficient patients might thus be beneficial.

The incretin system may play another crucial role in the metabolic control of type 3c diabetes mellitus. The regulation of the beta-cell mass and the physiological incretin secretion are directly dependent on normal exocrine pancreatic function and fat hydrolysis. Chronic pancreatitis and exocrine dysfunction have been associated with a functional impairment of the incretin system. Impaired GLP-1 secretion, however, can by normalized by pancreatic enzyme supplementation as previously described[3,45,46].

Adequate oral pancreatic enzyme replacement therefore seems very important in type 3c diabetes mellitus. Besides helping to control symptoms of steatorrhea, it also seems capable of preventing qualitative malnutrition and metabolic complications.

CONCLUSION

Type 3c diabetes mellitus is a clinically important disease with a prevalence of 5%-10% among all patients with diabetes mellitus. The prevalence and clinical importance of this condition has been underestimated and underappreciated in the past.

Most patients with type 3c diabetes mellitus suffer from chronic pancreatitis as the underlying disease. The prevalence of (subclinical) chronic pancreatitis might also been underestimated as some studies suggest. Recognizing a diabetic state in patients with known chronic pancreatitis is obligatory. Patients should undergo screening tests in order to detect hyperglycemia early. Fasting glucose, HbA_{1c} and 75 g oral glucose tolerance testing are appropriate diagnostic tools. When diagnosing diabetes mellitus in patients with chronic pancreatitis, physicians should be aware of the existence of type 3c diabetes mellitus and should classify this condition correctly as pancreatogenic diabetes or type 3c diabetes mellitus.

To identify a (subclinical) chronic pancreatitis as the underlying condition of patients with the established diagnosis of diabetes mellitus certainly is the greater challenge in everyday practice. This is due to the fact that most physicians are not aware of type 3c diabetes melli-tus und (subclinical chronic) pancreatitis does not necessarily present in a clinically impressive manner. A patient with unspecific gastrointestinal complaints and diabetes mellitus should therefore always prompt further diagnostics with regard to type 3c diabetes mellitus.

Identifying patients with type 3c diabetes is important since the endocrinopathy in type 3c diabetes is very complex and complicated by additional present comorbidities such as maldigestion and concomitant qualitative malnutrition. Specific diagnostic criteria are proposed above (Table 1). The failure to correctly diagnose type 3c diabetes mellitus leads to failure to implement an appropriate medical therapy. It is mandatory to treat pancreatic exocrine insufficiency in these patients even if clear clinical symptoms such as steatorrhea or gastrointestinal complaints are missing. Adequate pancreatic enzyme supplementation therapy might for once help preventing a lack of fat-soluble vitamins (especially vitamin D). Additionally it might exert beneficial effects on the impaired incretin release in patients with chronic pancreatitis. Furthermore one has to realize that type 3c diabetes mellitus due to chronic pancreatitis might be referred to as a pre-malignant condition since both diseases are well accepted risk factors for the development of pancreatic cancer.

REFERENCES

1 **Wang W**, Guo Y, Liao Z, Zou DW, Jin ZD, Zou DJ, Jin G, Hu XG, Li ZS. Occurrence of and risk factors for diabetes mellitus in Chinese patients with chronic pancreatitis. *Pancreas* 2011; **40**: 206-212 [PMID: 21404458 DOI: 10.1097/MPA.0b013e31820032ae]

2 **Malka D**, Hammel P, Sauvanet A, Rufat P, O'Toole D, Bardet P, Belghiti J, Bernades P, Ruszniewski P, Lévy P. Risk factors for diabetes mellitus in chronic pancreatitis. *Gastroenterology* 2000; **119**: 1324-1332 [PMID: 11054391 DOI: 10.1053/gast.2000.19286]

3 **Ebert R**, Creutzfeldt W. Reversal of impaired GIP and insulin secretion in patients with pancreatogenic steatorrhea following enzyme substitution. *Diabetologia* 1980; **19**: 198-204 [PMID: 6997121]

4 **Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.** Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003; **26** Suppl 1: S5-S20 [PMID: 12502614 DOI: 10.2337/diacare.26.2007.S5]

5 **American Diabetes Association.** Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2011; **34** Suppl 1: S62-S69 [PMID: 21193628 DOI: 10.2337/dc11-S062]

6 **Hardt PD**, Brendel MD, Kloer HU, Bretzel RG. Is pancreatic diabetes (type 3c diabetes) underdiagnosed and misdiagnosed? *Diabetes Care* 2008; **31** Suppl 2: S165-S169 [PMID: 18227480 DOI: 10.2337/dc08-s244]

7 **Ewald N**, Kaufmann C, Raspe A, Kloer HU, Bretzel RG, Hardt PD. Prevalence of diabetes mellitus secondary to pancreatic diseases (type 3c). *Diabetes Metab Res Rev* 2012; **28**: 338-342 [PMID: 22121010 DOI: 10.1002/dmrr.2260]

8 **Ganda O.** Secondary forms of diabetes. In: Kahn CR, Weir GC (editors). Joslin's Diabetes Mellitus. New York: Lea & Febiger, 1994

9 **Alberti KGMM.** Diabetes secondary to pancreatopathy: an example of brittle diabetes. In: Tiengo A, Alberti KGMM, Del Prato S, Vranic M (editors). Diabetes Secondary to Pancreatopathy. Proceedings of the Post EASD International Symposium on Diabetes Secondary to Pancreatopathy, Padova, 21–22 September 1987, International Congress Series 762. Amsterdam: Excerpta Medica, 1988: 211–214

10 **Abu-Bakare A**, Taylor R, Gill GV, Alberti KG. Tropical or malnutrition-related diabetes: a real syndrome? *Lancet* 1986; **1**: 1135-1138 [PMID: 2871387 DOI: 10.1016/S0140-6736(86)91846-5]

11 **Mohan V,** Pitchumoni C. Tropical chronic pancreatitis. In: Beger HG, Warshaw AL, Buechler MW, Carr-Locke DL, Neoptolemos JP, Russel C, Sarr MG (editors). The Pancreas. London: Blackwell Science, 1998: 688-697

12 **Cui Y**, Andersen DK. Pancreatogenic diabetes: special considerations for management. *Pancreatology* 2011; **11**: 279-294 [PMID: 21757968 DOI: 10.1159/000329188]

13 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244 DOI: 10.1053/gast.2001.22586]

14 **Doerr W**. Pathogenesis of acute and chronic pancreatitis. *Verh Dtsch Ges Inn Med* 1964; **70**: 718-758 [PMID: 14298752 DOI: 10.1007/978-3-642-96030-7_116]

15 **Olsen TS**. Lipomatosis of the pancreas in autopsy material and its relation to age and overweight. *Acta Pathol Microbiol Scand A* 1978; **86A**: 367-373 [PMID: 716899 DOI: 10.1111/j.1699-0463.1978.tb02058.x]

16 **Blumenthal HT**, Probstein JG, Berns AW. Interrelationship of diabetes mellitus and pancreatitis. *Arch Surg* 1963; **87**: 844-850 [PMID: 14058852 DOI: 10.1001/archsurg.1963.01310170130022]

17 **Petrone MC**, Arcidiacono PG, Perri F, Carrara S, Boemo C, Testoni PA. Chronic pancreatitis-like changes detected by endoscopic ultrasound in subjects without signs of pancreatic disease: do these indicate age-related changes, effects of xenobiotics, or early chronic pancreatitis? *Pancreatology* 2010; **10**: 597-602 [PMID: 20980777 DOI: 10.1159/000314599]

18 **Rothenbacher D**, Löw M, Hardt PD, Klör HU, Ziegler H, Brenner H. Prevalence and determinants of exocrine pancreatic insufficiency among older adults: results of a population-based study. *Scand J Gastroenterol* 2005; **40**: 697-704 [PMID: 16036530 DOI: 10.1080/00365520510023116]

19 **Rebours V**, Boutron-Ruault MC, Schnee M, Férec C, Le Maréchal C, Hentic O, Maire F, Hammel P, Ruszniewski P, Lévy P. The natural history of hereditary pancreatitis: a national series. *Gut* 2009; **58**: 97-103 [PMID: 18755888 DOI: 10.1136/gut.2008.149179]

20 **Howes N**, Lerch MM, Greenhalf W, Stocken DD, Ellis I, Simon P, Truninger K, Ammann R, Cavallini G, Charnley RM, Uomo G, Delhaye M, Spicak J, Drumm B, Jansen J, Mountford R, Whitcomb DC, Neoptolemos JP. Clinical and genetic characteristics of hereditary pancreatitis in Europe. *Clin Gastroenterol Hepatol* 2004; **2**: 252-261 [PMID: 15017610 DOI: 10.1016/S1542-3565(04)00013-8]

21 **Rickels MR**, Bellin M, Toledo FG, Robertson RP, Andersen DK, Chari ST, Brand R, Frulloni L, Anderson MA, Whitcomb DC. Detection, evaluation and treatment of diabetes mellitus in chronic pancreatitis: recommendations from PancreasFest 2012. *Pancreatology* 2013; **13**: 336-342 [PMID: 23890130 DOI: 10.1016/j.pan.2013.05.002]

22 **Ewald N**, Bretzel RG. Diabetes mellitus secondary to pancreatic diseases (Type 3c)--are we neglecting an important

disease? *Eur J Intern Med* 2013; **24**: 203-206 [PMID: 23375619 DOI: 10.1016/j.ejim.2012.12.017]

23 **Hardt PD**, Krauss A, Bretz L, Porsch-Ozcürümez M, Schnell-Kretschmer H, Mäser E, Bretzel RG, Zekhorn T, Klör HU. Pancreatic exocrine function in patients with type 1 and type 2 diabetes mellitus. *Acta Diabetol* 2000; **37**: 105-110 [PMID: 11277309 DOI: 10.1007/s005920070011]

24 **Noel RA**, Braun DK, Patterson RE, Bloomgren GL. Increased risk of acute pancreatitis and biliary disease observed in patients with type 2 diabetes: a retrospective cohort study. *Diabetes Care* 2009; **32**: 834-838 [PMID: 19208917 DOI: 10.2337/dc08-1755]

25 **Moriai T**, Morita Y, Matsui T, Okada M. Type 1 diabetes mellitus associated with clinical acute pancreatitis in an adult. *Pancreas* 2000; **20**: 415-416 [PMID: 10824699 DOI: 10.1097/00006676-200005000-00014]

26 **Larsen S**, Hilsted J, Tronier B, Worning H. Pancreatic hormone secretion in chronic pancreatitis without residual beta-cell function. *Acta Endocrinol* (Copenh) 1988; **118**: 357-364 [PMID: 2899369 DOI: 10.1530/acta.0.1180357]

27 **Valenzuela JE**, Taylor IL, Walsh JH. Pancreatic polypeptide response in patients with chronic pancreatitis. *Dig Dis Sci* 1979; **24**: 862-864 [PMID: 520107 DOI: 10.1007/BF01324903]

28 **Glaser B**, Zoghlin G, Pienta K, Vinik AI. Pancreatic polypeptide response to secretin in obesity: effects of glucose intolerance. *Horm Metab Res* 1988; **20**: 288-292 [PMID: 3042579 DOI: 10.1055/s-2007-1010817]

29 **Larsen S**. Diabetes mellitus secondary to chronic pancreatitis. *Dan Med Bull* 1993; **40**: 153-162 [PMID: 8495594]

30 **Larsen S**, Hilsted J, Tronier B, Worning H. Metabolic control and B cell function in patients with insulin-dependent diabetes mellitus secondary to chronic pancreatitis. *Metabolism* 1987; **36**: 964-967 [PMID: 3309547]

31 **The Diabetes Control and Complications Trial (DCCT)**. Design and methodologic considerations for the feasibility phase. The DCCT Research Group. *Diabetes* 1986; **35**: 530-545 [PMID: 2869996 DOI: 10.2337/diabetes.35.5.530]

32 **UK Prospective Diabetes Study (UKPDS)**. VIII. Study design, progress and performance. *Diabetologia* 1991; **34**: 877-890 [PMID: 1778353 DOI: 10.1007/BF00400195]

33 **Nathan DM**, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009; **32**: 193-203 [PMID: 18945920 DOI: 10.2337/dc08-9025]

34 **Sadeghi N**, Abbruzzese JL, Yeung SC, Hassan M, Li D. Metformin use is associated with better survival of diabetic patients with pancreatic cancer. *Clin Cancer Res* 2012; **18**: 2905-2912 [PMID: 22465831 DOI: 10.1158/1078-0432.CCR-11-2994]

35 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437 [PMID: 8479461 DOI: 10.1056/NEJM199305203282001]

36 **Magruder JT**, Elahi D, Andersen DK. Diabetes and pancreatic cancer: chicken or egg? *Pancreas* 2011; **40**: 339-351 [PMID: 21412116 DOI: 10.1097/MPA.0b013e318209e05d]

37 **Duell EJ**, Lucenteforte E, Olson SH, Bracci PM, Li D, Risch HA, Silverman DT, Ji BT, Gallinger S, Holly EA, Fontham EH, Maisonneuve P, Bueno-de-Mesquita HB, Ghadirian P, Kurtz RC, Ludwig E, Yu H, Lowenfels AB, Seminara D, Petersen GM, La Vecchia C, Boffetta P. Pancreatitis and pancreatic cancer risk: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann Oncol* 2012; **23**: 2964-2970 [PMID: 22767586 DOI: 10.1093/annonc/mds140]

38 **Alves C**, Batel-Marques F, Macedo AF. A meta-analysis of serious adverse events reported with exenatide and liraglutide: acute pancreatitis and cancer. *Diabetes Res Clin Pract* 2012; **98**: 271-284 [PMID: 23010561 DOI: 10.1016/j.diabres.2012.09.008]

39 **Klapdor S**, Richter E, Klapdor R. Vitamin D status and peroral vitamin D supplementation in patients suffering from chronic pancreatitis and pancreatic cancer disease. *Anticancer Res* 2012; **32**: 1991-1998 [PMID: 22593477]

40 **Sikkens EC**, Cahen DL, Koch AD, Braat H, Poley JW, Kuipers EJ, Bruno MJ. The prevalence of fat-soluble vitamin deficiencies and a decreased bone mass in patients with chronic pancreatitis. *Pancreatology* 2013; **13**: 238-242 [PMID: 23719594 DOI: 10.1016/j.pan.2013.02.008]

41 **Mann ST**, Mann V, Stracke H, Lange U, Klör HU, Hardt P, Teichmann J. Fecal elastase 1 and vitamin D3 in patients with osteoporotic bone fractures. *Eur J Med Res* 2008; **13**: 68-72 [PMID: 18424365]

42 **Teichmann J**, Mann ST, Stracke H, Lange U, Hardt PD, Klör HU, Bretzel RG. Alterations of vitamin D3 metabolism in young women with various grades of chronic pancreatitis. *Eur J Med Res* 2007; **12**: 347-350 [PMID: 17933711]

43 **Ford ES**, Zhao G, Tsai J, Li C. Associations between concentrations of vitamin D and concentrations of insulin, glucose, and HbA1c among adolescents in the United States. *Diabetes Care* 2011; **34**: 646-648 [PMID: 21273498 DOI: 10.2337/dc10-1754]

44 **Boucher BJ**. Vitamin D insufficiency and diabetes risks. *Curr Drug Targets* 2011; **12**: 61-87 [PMID: 20795936 DOI: 10.2174/138945011793591653]

45 **Beglinger S**, Drewe J, Schirra J, Göke B, D'Amato M, Beglinger C. Role of fat hydrolysis in regulating glucagon-like Peptide-1 secretion. *J Clin Endocrinol Metab* 2010; **95**: 879-886 [PMID: 19837920 DOI: 10.1210/jc.2009-1062]

46 **Kuo P**, Stevens JE, Russo A, Maddox A, Wishart JM, Jones KL, Greville H, Hetzel D, Chapman I, Horowitz M, Rayner CK. Gastric emptying, incretin hormone secretion, and postprandial glycemia in cystic fibrosis--effects of pancreatic enzyme supplementation. *J Clin Endocrinol Metab* 2011; **96**: E851-E855 [PMID: 21389144 DOI: 10.1210/jc.2010-2460]

P- Reviewers: Dumitrascu DL, Sakata N **S- Editor:** Gou SX
L- Editor: A **E- Editor:** Wu HL

WJG
World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7282

World J Gastroenterol 2013 November 14; 19(42): 7282-7291
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Pain and chronic pancreatitis: A complex interplay of multiple mechanisms

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Received: June 18, 2013 **Revised:** July 22, 2013
Accepted: August 20, 2013
Published online: November 14, 2013

Abstract

Despite multiple theories on the pathogenesis of pain in chronic pancreatitis, no uniform and consistently successful treatment strategy exists and abdominal pain still remains the dominating symptom for most patients and a major challenge for clinicians. Traditional theories focussed on a mechanical cause of pain related to anatomical changes and evidence of increased ductal and interstitial pressures. These observations form the basis for surgical and endoscopic drainage procedures, but the outcome is variable and often unsatisfactory. This underscores the fact that other factors must contribute to pathogenesis of pain, and has shifted the focus towards a more complex neurobiological understanding of pain generation. Amongst other explanations for pain, experimental and human studies have provided evidence that pain perception at the peripheral level and central pain processing of the nociceptive information is altered in patients with chronic pancreatitis, and resembles that seen in neuropathic and chronic pain disorders. However, pain due to *e.g.*, complications to the disease and adverse effects to treatment must not be overlooked as an additional source of pain. This review outlines the current theories on pain generation in chronic pancreatitis which is crucial in order to understand the complexity and limitations of current therapeutic approaches. Furthermore, it may also serve as an inspiration for further research and development of methods that can evaluate the relative contribution and interplay of different pain mechanisms in the individual patients, before they are subjected to more or less empirical treatment.

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Key words: Chronic pancreatitis; Abdominal pain; Pain mechanisms; Neuropathy; Sensitization

Core tip: Pain management in chronic pancreatitis often remains unsatisfactory. An overview of the current theories on pain generation in chronic pancreatitis is crucial in order to understand the complexity and limitations of current therapeutic approaches. Also, optimal treatment will only be achieved on the basis of a better understanding of these mechanisms. Furthermore, this review may serve as an inspiration for future research and development of methods that can determine the relative contribution of different mechanisms to the "collective" abdominal pain, before patients are subjected to more or less empirical treatment.

Poulsen JL, Olesen SS, Malver LP, Frøkjær JB, Drewes AM. Pain and chronic pancreatitis: A complex interplay of multiple mechanisms. *World J Gastroenterol* 2013; 19(42): 7282-7291 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7282.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7282

INTRODUCTION

Abdominal pain is the most significant symptom and a major clinical challenge in chronic pancreatitis (CP). It is present in up to 90% of the patients and the primary cause of hospitalization[1]. Pancreatic pain is characteristically described as a constant, severe, dull, epigastric pain that often radiates to the back and typically worsens after high-fat meals. However, many different pain patterns have been described, ranging from no pain to recurrent episodes of pain and pain free intervals, to constant pain with clusters of severe exacerbations[2,3]. In the often cited Zürich series, pain was reported to decrease over time, coinciding with the occurrence of exocrine insufficiency, which led to the "burn-out" hypothesis of pain in CP[3,4]. However, evidence against this hypothesis was subsequently provided in two large prospective studies[5,6], where no association between the duration of CP and the quality or frequency of pain was found. Today the "burn-out" hypothesis is regarded obsolete by most clinicians, and even though a few patients may experience spontaneously pain relief, pain in the majority of patients is unpredictable and has a substantial impact on physical and mental quality of life, employment and health care expenses[6-8].

The etiology of pain in CP is increasingly better understood and likely involves multiple mechanisms. Traditional theories focused on the role of local pathology within or in close proximity to the pancreas, as the main generator of pain, but since the late 1990's there has been a shift towards a neurobiological understanding of pain in CP. Accordingly, there is solid histological and neurophysiological evidence for an abnormal pain processing in many of these patients[1,9]. Various mechanisms responsible for this altered pain processing have been proposed, among them sensitization of the peripheral and central nerves, reorganization of the cerebral cortex and alterations in pain control systems. In addition, local complications (such as pancreatic pseudocysts and duodenal and/or bile duct obstruction) and adverse effects to treatment also contribute to the complex symptomatology in many patients and is an often a neglected problem in the clinical settings. Also, it has been shown, that the clinical pain pattern varies with the etiology of CP, and hypothesized that different pathogenic mechanisms of pain are associated with or predominates depending on etiology[2,4], but so far it is not possible to correlate pain mechanism and clinical pain pattern to the etiology of CP uniformly.

The aim of this review is to highlight current theories regarding the pain mechanisms in CP. This is important in order to understand the complexity of this debilitating disease and why pain management is often insufficient. Also, optimal treatment will only be achieved on the basis of a better understanding of the mechanisms underlying pain in CP[10]. There is a variety of potential pain mechanisms that needs to be taken into consideration (Figure 1), but in this review we present for simplicity a schematic division of the pain mechanisms into five main categories as an easy-to-remember overview (Table 1).

"PLUMBING" PROBLEMS

The mainstay of pain treatment in CP has for many years focused on the pancreatic gland based on the assumption, that pain is generated by increased pressure in the pancreatic duct or in the pancreatic parenchyma[11]. This mechanistic understanding of pain, termed the "the plumbing theory", has been the most widely accepted theory regarding the cause of pain and it is the theoretical background of most interventions including surgical and endoscopic drainage procedures[12].

Pancreatic duct hypertension and pain

A direct relationship between pancreatic duct hypertension and pain was reported for the first time in a case report by White *et al*[13]. A patient undergoing open necrosectomy following acute necrotizing pancreatitis had a drainage catheter placed in a fistula tract communicating with the pancreatic duct. He reproducibly developed pain after saline infusion when the ductal pressure exceeded 25 mmHg[13]. Inspired by this finding, subsequent studies attempted to compare ductal pressure measurements in CP patients and other patient groups. Many of these studies were flawed by inappropriate comparison of intraoperative pressure measurements in CP patients to endoscopic pressure measurement in controls. Accordingly, only a few studies have compared ductal pressure using the same technique in patients and controls. Based on intraoperative measurement of pancreatic duct pressure, significantly increased pressures were evident in patients with CP undergoing a surgical pancreatic drainage procedure compared to patients undergoing surgery for gastric cancer[14]. Moreover, in a study comparing endoscopic manometry of the pancreatic duct, CP patients had evidence of ductal hypertension compared to controls[15]. Other studies based on endoscopic manometry have, however, not documented ductal hypertension in CP, and the only study comparing CP patients by the presence or absence of pain demonstrated no difference in pressure levels[16-20]. In addition, the pathological mechanism by which increased ductal pressure causes pain in CP is not clear and, taken together, the link between ductal hypertension and pain in CP remains hypothetical.

Pancreatic parenchymal hypertension and pain

Measurement of intrapancreatic pressure was pioneered by Ebbehøj *et al*[21], who developed a technique based on a needle probe inserted directly into the pancreatic parenchyma. Measurements were employed directly intraoperatively or by using a percutaneous procedure guided by ultrasonography[21]. In 39 patients with CP, they found that the intrapancreatic pressure was higher in patients

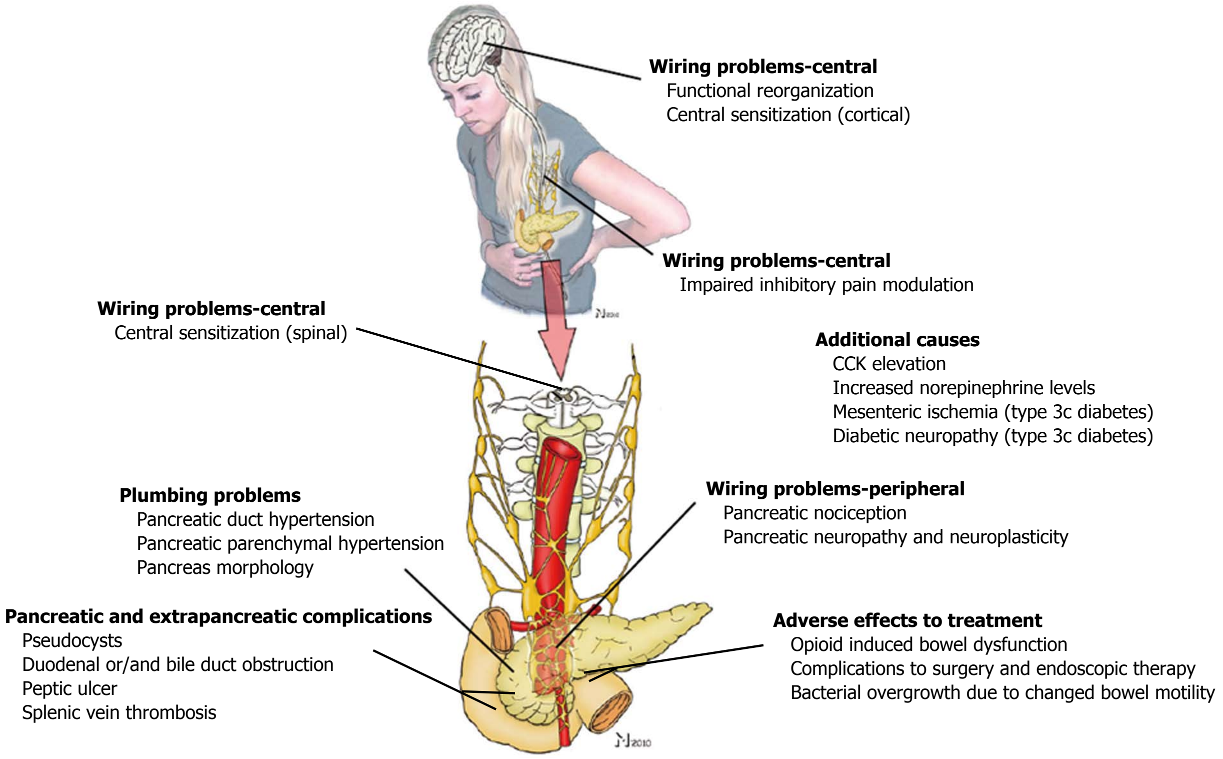
Wiring problems-central
Functional reorganization
Central sensitization (cortical)
Wiring problems-central
Impaired inhibitory pain modulation
Wiring problems-central
Central sensitization (spinal)
Additional causes
CCK elevation
Increased norepinephrine levels
Mesenteric ischemia (type 3c diabetes)
Diabetic neuropathy (type 3c diabetes)
Plumbing problems
Pancreatic duct hypertension
Pancreatic parenchymal hypertension
Pancreas morphology
Wiring problems-peripheral
Pancreatic nociception
Pancreatic neuropathy and neuroplasticity
Pancreatic and extrapancreatic complications
Pseudocysts
Duodenal or/and bile duct obstruction
Peptic ulcer
Splenic vein thrombosis
Adverse effects to treatment
Opioid induced bowel dysfunction
Complications to surgery and endoscopic therapy
Bacterial overgrowth due to changed bowel motility
MJ2010

Figure 1 Pain mechanisms in chronic pancreatitis. The source of pain in chronic pancreatitis is complex and almost certainly multifactorial, and the relative contribution of different factors can be difficult to determine in the individual patient. See manuscript for further details. Modified from Demir *et al*[87]. CCK: Cholecystokinin.

with pain than in patients without pain, and this intrapancreatic hypertension was reversed following surgical drainage[22]. At one-year follow-up, patients with recurrent pain had a rebound of intrapancreatic hypertension, while patients who remained pain free continued to have normal intrapancreatic pressures[23]. Although intriguing, these findings were not reproduced by a subsequent study in which no relation between intrapancreatic pressure and pain was found[24].

The pathophysiological link between intrapancreatic hypertension and pain has been explained as a "compartment-like syndrome" induced by fibrosis of the pancreatic parenchyma and peripancreatic capsule[2]. During secretory stimulation in an animal model of CP, increased interstitial pressures, diminished blood flow and ensuing tissue acidosis were documented[25]. The latter was possibly secondary to ischemia, thus mimicking the pathophysiology underlying muscular compartment syndrome. Although no pain data were collected in this experiment, it is plausible that acidosis may have caused pain by activation of the transient receptor potential vanilloid-1, which is a nociceptor found to be up regulated in CP and other conditions characterized by neurogenic inflammation[26,27] (described further below). However, it must be highlighted that these findings have never been reproduced in a human study and needs confirmation.

Pancreas morphology and pain

In clinical practice, measurement of ductal or intrapancreatic pressure is not routinely performed and most decisions regarding surgery or endotherapy to relieve pain rely on morphological abnormalities of the pancreas[2,11,28]. However, the relationship between ductal or intrapancreatic pressure and pancreatic morphology is ambiguous[29]. Furthermore, in a very recent study by Frokjaer *et al*[30] magnetic resonance cholangiopancreatography including diffusion weighted imaging was obtained in 23 patients with painful CP and 17 controls. Compared to traditional imaging techniques (*i.e.*, endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography, computed tomography, and endoscopic ultrasound), which mainly focus on atrophy and ductal pathology, this modality also provides a measure of fibrotic changes in the pancreatic gland[31]. Interestingly, the authors found no association between degree of pathological imaging (fibrosis, atrophy, and ductal pathology) and pain. However, pancreatic atrophy and ductal pathology were associated with diabetes (*i.e.*, more severe atrophy in patients with high levels of glycated hemoglobin), and low levels of phosphate and hemoglobin.

Taken together, pathological pancreatic morphology does not necessarily reflect the burden of pain in CP. Nowhere is this observation made more obvious, than by the patient who undergoes total pancreatectomy for painful CP and still continues to suffer from pain[11].

"WIRING" PROBLEMS

Lesions to intrapancreatic nerves and their impact on the pathogenesis of pain in CP have been the focus of

Table 1 Pain mechanisms in chronic pancreatitis

Main category	Proposed mechanism/source	Ref.
Plumbing problems	Pancreatic duct hypertension	[14,16,20]
	Pancreatic parenchymal hypertension	[21-23]
	Pancreas morphology	[29,30]
Wiring problems	Peripheral nociception	[35-37]
	Pancreatic neuropathy	[41,42,44]
	Central mechanisms of pain	[50,52,53,56]
Pancreatic and extrapancreatic complications	Pseudocysts	[62,64]
	Duodenal obstruction	[66]
	Bile duct obstruction	[66,67]
	Peptic ulcer	[71,72]
	Splenic vein thrombosis	[75,78]
Adverse effects to treatment	Opioid induced bowel dysfunction	[79]
	Complications to surgery and endoscopic therapy	[12]
	Bacterial overgrowth due to changed bowel motility	[81]
Additional causes	CCK elevation	[82]
	Increased norepinephrine levels	[85]
	Diabetic neuropathy (type 3c diabetes)	[86]
	Mesenteric ischemia[1]	

[1]Complications to and/or possible concomitant diseases. CCK: Cholecystokinin.

a substantial amount of research past 25 years. The International Association for the Study of Pain recently changed its definition of neuropathic pain to "pain caused by a lesion or disease of the somatosensory system"[32]. In the context of pain and CP there is emerging histological and neurophysiological evidence of such lesions to peripheral nerves in the pancreatic gland and coincident aberrant central pain processing. Three aspects of the neural basis of pain in CP are usually considered: Peripheral nociception, pancreatic neuropathy and central mechanisms of pain.

Peripheral nociception

A nociceptor is a sensory nerve cell capable of integrating and transducing nociceptive stimuli into pain signals[33]. Nociception begins with the primary afferent nerves, whose cell bodies lies in the dorsal root ganglia next to the spinal cord. They have two branches-one terminating in the target tissue and one that ends in the dorsal horn of the spinal cord. The peripheral nerve endings sense tissue injury or pain stimuli *via* a variety of receptors, which respond to physical or chemical factors in their surroundings[12]. Depending on the excitability of the neural membrane, the stimulus leads to an action potential, which travels to the spinal end of the nerves in the dorsal horn. Here it triggers the release of neurotransmitters, which cross the synapse and act on secondary neurons and transmit the stimulus to the brain through diverse pathways, ultimately resulting in the sensation of pain.

Currently, it is unknown what actually activates the intrapancreatic nociceptors in humans. However, increased expression of the transient receptor potential vanilloid-1 has been demonstrated in an animal model of CP[34], as well as in humans, although no correlation to pain was seen[35]. Furthermore, studies on nociceptive activation markers, *i.e.*, neurotransmitters secreted by activated afferents, especially the large family of neurotrophic factors, are gaining increasing attention. Among others, both nerve growth factor and brain-derived neurotrophic factor have been shown to be upregulated in CP patients[36,37], as well as many other proinflammatory cytokines, and in some cases this upregulation has been associated with increased pain intensity and/or frequency (for a review see[2]).

These changes render the nociceptors more sensitive to further stimulation, so that there can be a reduction in the threshold for activation, an increase in the response to a given stimulus, or the appearance of spontaneous activity[38,39]. This sensitization, called peripheral sensitization, results in an increased barrage of pain signals to the spinal cord[40].

Taken together, several articles have demonstrated upregulation signaling molecules involved in inflammation and pronociceptive mediators, but also neurotrophic factors in CP. The trophic nature of the nociceptive activation markers further suggests a link between this altered nociception and the neuropathy of CP described below.

Pancreatic neuropathy

Increased neural density and hypertrophy, sprouting and neuritis of the intrapancreatic nerves, as well as activation of glia and immune cells have also been reported in pancreatic tissue from CP patients[41-43]. These changes, collectively known as neuropathy, have been strongly associated with clinical pain scores, and thus suggests to be an important factor in the pathogenesis of pain in CP[41,42,44]. Autonomic innervation, especially sympathetic, is also decreased[43], and has been correlated with abdominal pain.

Overall, substantial evidence supports the notion that pancreatic neuropathy leads to a remodeling of the intrapancreatic innervation, a concept known as pancreatic neuroplasticity, which is likely an important factor in the pathogenesis of pain in CP.

Central mechanisms of pain

A sustained and increased peripheral nociceptive drive may result in an increased responsiveness of central pain transmitting neurons. This phenomenon is known as central sensitization, and refers to an increased synaptic efficiency established in sensory neurons in the dorsal horn of the spinal cord (and/or at supraspinal sites), following intense peripheral noxious stimuli, tissue injury, or nerve damage[45,46]. The end result is pain, which is no longer coupled to the presence, intensity, or duration of noxious peripheral stimuli[47]. Clinically these changes manifests as allodynia (a painful response to stimuli not normally painful), hyperalgesia (increased sensitivity to painful stimuli) and secondary hyperalgesia (a receptive field expansion that enables input from non-injured tissue to produce pain)[45].

Several studies have reported findings compatible with central sensitization in CP. Patterns of referred pain can be examined to assess central sensitization, as viscera-somatic convergence between peripheral nerves occurs at the spinal cord and higher levels of the central nervous system. Hence, visceral sensitivity of the upper gastrointestinal organs (sharing spinal innervation with the pancreatic gland) serves as a proxy of spinal sensitization[48,49]. In one study, increased areas of referred pain to electrical stimulation of the esophagus, stomach, and duodenum was reported in CP patients compared to controls[50]. Other studies reported decreased pain thresholds to visceral stimulation of the rectosigmoid as well as somatic stimulation of muscle and bone[51,52]. Taken together, these findings characterize a generalized hyperalgesic state of the pain system and likely mirrors widespread sensitization of the central nervous system as seen in many other chronic pain disorders[45].

From the spinal cord, visceral afferents are projected to cortical and subcortical structures of the brain. Experimental pain studies, based on somatic stimulation of the epigastric skin area (sharing spinal segmental innervation with the pancreatic gland) as well as visceral stimulation of the upper and lower gut with concomitant recording of evoked brain potentials and brain source localization, have indicated that chronic pain and hyperalgesia is associated with functional reorganization of the cerebral cortex[50,53-55]. Hence, compared to healthy controls CP patients show reorganization of the brain areas involved in visceral pain processing including the insula, secondary somatosensory cortex and cingulate cortex similar to what is seen in phantom pain[53]. Interestingly, insular reorganization was associated with clinical pain intensity. In addition to reorganization of the brain areas involved in visceral pain processing, the excitability of these neural networks is abnormal with evidence of impaired habituation to noxious stimuli, possibly reflecting a neuronal hyperexcitability (*i.e.*, cortical sensitization)[55].

The structural correlate of functional cortical reorganization and hyperexcitability is found in studies based on advanced magnetic resonance imaging (MRI). In one study using diffusion weighted MRI, microstructural changes in the insular and frontal brain areas was associated with clinical pain intensity and functional scores[56]. Patients with a constant pain pattern demonstrated the most severe microstructural abnormalities compared to patients with an attack-wise pain pattern. This translates well to the clinic where patients with constant pain was recently reported to have the most reduced quality of life and functioning[6]. In another MRI study based on cortical volumetry, brain areas involved in visceral pain processing was shown to have a reduced thickness[57]. This finding suggests a central neurodegenerative response to severe and sustained pain.

The pain system has several inherent mechanisms whereby inflowing pain signals can be modulated. In particular, modulation of spinal pain transmission from the brainstem and higher cortical areas has been subject to increased interest during the last decades. Descending pain modulation where the brain can exert a downstream gating of the incoming spinal activity can lead to either an increase in the spinal transmission of pain impulses (facilitation) or a decrease in transmission (inhibition). The balance between these states ultimately determines the quality and strength of the pain signals perceived by the brain. Alterations in the state of descending modulation from inhibition towards facilitation have been implicated in the transition of acute into chronic and neuropathic pain. In the context of pain and CP, impaired descending inhibitory pain modulation has been reported in studies based on experimental human pain models[52,58]. In addition, brainstem facilitation has been reported to maintain pancreatic pain in an animal model of CP[59].

Taken together, several lines of evidence indicate that central pain processing is abnormal in CP. However, it is difficult to determine whether these central abnormalities are maintained by a sustained nociceptive drive from the pancreatic gland or whether they have become independent of peripheral input. In favor of the latter, a recent pilot project documented generalized hyperalgesia (a clinical measurable proxy of central sensitization) to be associated with failure of thoracoscopic splanchnic denervation[60]. The authors concluded that in patients with hyperalgesia and failure to denervation, the disease has advanced and the generation of pain becomes self-perpetuating and independent of the initial peripheral nociceptive drive. However, these findings need confirmation in larger and longitudinal studies.

PANCREATIC AND EXTRAPANCREATIC COMPLICATIONS

Although questions still remain, “plumbing” and “wiring” problems are probably important generators of pain in CP. Nevertheless, pain due to complications to the disease is also likely to contribute, and should not be overlooked, when evaluating the origin of abdominal pain in CP patients.

Pseudocysts

Pancreatic pseudocysts are relatively common in CP with estimated incidence rates of 20%-40%[61,62], however, there is a lack of precise data based on long term follow-up. Yet, due to the chronic nature of the disease, CP patients are at high risk of developing pseudocyst in the course of their disease[63]. The exact pathogenesis of pseudocyst formation in CP is not known, but it has been proposed, that blockage of the major branch of the main pancreatic duct, and ongoing pancreatic secretion proximal to the obstruction leads to a saccular dilatation of the duct. This is then filled with pancreatic juice resulting in a pseudocyst[62]. The range of symptoms is wide-from asymptomatic to severe abdominal pain dependent on etiology, localization, and size[64,65].

Duodenal and bile duct obstruction

Advanced CP is characterized by replacement of the pancreatic parenchyma with fibrous tissue. Due to the close anatomical relationship of the common bile duct and the second part of the duodenum with the head of the pancreas, fibrosis can exert extrinsic pressure on these structures. The end result is mechanical obstruction of the common bile duct and duodenum[66,67].

The clinical presentation of duodenal and bile duct obstruction is variable and can be asymptomatic[66,68]. Postprandial abdominal pain, early satiety and nausea are the most common symptoms of duodenal obstruction, while pain and abnormal liver function tests are suggestive of bile duct stenosis and cholangitis[69]. The mechanisms underlying such "obstructive pain" are unclear and one study concluded that bile duct obstruction without cholangitis is not the cause of pain in patients with CP[70].

Peptic ulcer

Upper abdominal pain is a prominent symptom in patients with peptic ulcer. Previous studies have demonstrated, that the prevalence of duodenal ulcer is high in patients with CP (ranges from 3.6% to 37.5%)[5,71,72]. The reason for the higher prevalence is unclear and many hypotheses have been suggested, including higher prevalence of *Helicobacter pylori* infection[71], increased gastric acid secretion[73,74], and insufficient pancreatic exocrine function which reduces bicarbonate secretion and decreases duodenal pH[72]. Furthermore, changes in gastric and intestinal blood flow due to previous acute pancreatitis may also be of importance.

As peptic ulcer can be asymptomatic, the high prevalence may be a result of a "detection bias" due to intensive search for the "origin" of epigastric pain in a selected group of patients. Patients with CP are more likely to undergo an upper gastrointestinal endoscopy, and therefore ulcers with atypical or no symptoms may be included, causing a higher prevalence than in the background population[72].

Under all circumstances, peptic ulcer may contribute to or be the reason for abdominal pain in CP patients and therefore diagnostic upper gastrointestinal endoscopy should be considered on wide indications.

Splenic vein thrombosis

The splenic vein runs along the posterior surface of the pancreas and may be affected by inflammation in the pancreatic gland, leading to thrombosis and abdominal pain. Because most patients are asymptomatic the incidence of splenic vein thrombosis in CP is not known[75], but incidence from 4%[76] to 45%[77] have been reported. Only few studies address the issue, but in one of the largest studies on splenic vein thrombosis in CP, 266 patients were investigated prospectively for a mean time of 8.2 years[78]. Splenic vein thrombosis was found in 22 patients (8.3%), but was only symptomatic in two patients. Hence, the role of splenic vein thrombosis in the pathogenesis of pain in CP is still questionable.

ADVERSE EFFECTS TO TREATMENT

In many CP patients, strong opioids are often necessary to relieve pain. Unfortunately, opioids have the potential to produce substantial gastrointestinal adverse effects, including constipation, reflux, nausea and abdominal pain-a phenomenon known as opioid-induced bowel dysfunction[79]. In a study on the prevalence of gastrointestinal symptoms in patients treated with opioids for non-cancerous diseases, chronic abdominal pain was reported in 58% of the patients[80]. Hence, opioid consumption may confuse the clinical picture in CP and worsen or contribute significantly to abdominal pain. Medications that change bowel motility may also contribute to the bacterial overgrowth that is reported in up to 40% of the patients[81]. This may manifest as abdominal distension and pain.

In addition to side effects to medical therapy, complications to surgical end endoscopic therapy may cause pain in a number of patients. These may include postoperative adhesions, ductal and parenchymal trauma, pancreatic and bile duct strictures, and complications to pancreatic stenting-all of which may result in abdominal pain. However, the relative contribution to abdominal pain due to surgical complications in CP is difficult to assess, and as far as the authors are aware of, no studies have examined this.

ADDITIONAL CAUSES FOR PAIN

The above mentioned theories and mechanisms of pain are considered the most common and well-founded by the authors, but other rarer, less well examined causes, and complications to concomitant and comorbid diseases may also play in role in pain generation. A thorough evaluation of all these plausible causes is beyond the scope of this review, but the reader is referred Table 1 for an overview.

However, elevated cholecystokinin (CCK) levels and increased sympathetic activity deserves to be mentioned separately as an additional cause of pain in CP. It has been shown, that CCK levels are elevated threefold in some early pancreatitis patients compared to controls[82]. This may generate pain by increasing the pressure in the pancreatic duct, but also through direct activation of nociceptive pathways in the central nervous system[83]. In one placebo-controlled, multicentre trial including 207 patients with chronic pancreatitis, an oral CCK receptor antagonist significantly decreased pain over placebo, although the placebo response was 30%[84]. However, further research into CCK antagonists has been hampered by concerns about induction of exocrine insufficiency and gallstones[11].

Although limited by a small number of patients, Buscher *et al*[85] showed significantly lower pain tolerance thresholds to pressure pain in dermatome T10 (pancreatic dermatome) in CP patients with increased norepinephrine levels compared to CP patients with normal norepinephrine levels, suggesting that sympathetic activity may play a role in these patients pain processing.

CONCLUSION

Intense abdominal pain is a dominant feature of CP and it is associated with poor mental and physical quality of life. Basic studies of pancreatic nerves and experimental human pain research have provided evidence that pain processing is abnormal in these patients and in many cases resembles that seen in neuropathic and chronic pain disorders. This neurobiological view of pain is somewhat in opposition to the traditional view of pain etiology in CP, where pain was assumed to arise from pathology in or in close proximity to the pancreatic gland. However, these theories are not mutually exclusive, and aspects of both may contribute in the generation and perpetuation of pain. In addition, adverse effects and complications to medical and interventional therapies may account for a substantial morbidity in many patients and should be considered as an additional source of pain. Therefore, it is important to consider the different mechanisms, when evaluating the origin of pain in CP patients (Figure 1), and it is plausible that the "collective" abdominal pain is a result of a complex interplay of several mechanisms. This novel and multifaceted understanding of pain etiology in CP requires a paradigm shift in pain management. Hence, modern mechanism based pain treatments taking into account especially altered pain processing are likely to increasingly replace traditional invasive therapies. In addition, the improved understanding of pain etiology will likely pave the way for new treatment modalities.

REFERENCES

1 **Pasricha PJ**. Unraveling the mystery of pain in chronic pancreatitis. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 140-151 [PMID: 22269952 DOI: 10.1038/nrgastro.2011.274]

2 **Fasanella KE**, Davis B, Lyons J, Chen Z, Lee KK, Slivka A, Whitcomb DC. Pain in chronic pancreatitis and pancreatic cancer. *Gastroenterol Clin North Am* 2007; **36**: 335-64, ix [PMID: 17533083 DOI: 10.1016/j.gtc.2007.03.011]

3 **Ammann RW**, Muellhaupt B. The natural history of pain in alcoholic chronic pancreatitis. *Gastroenterology* 1999; **116**: 1132-1140 [PMID: 10220505]

4 **Ammann RW**, Buehler H, Muench R, Freiburghaus AW, Siegenthaler W. Differences in the natural history of idiopathic (nonalcoholic) and alcoholic chronic pancreatitis. A comparative long-term study of 287 patients. *Pancreas* 1987; **2**: 368-377 [PMID: 3628234]

5 **Lankisch PG**, Löhr-Happe A, Otto J, Creutzfeldt W. Natural course in chronic pancreatitis. Pain, exocrine and endocrine pancreatic insufficiency and prognosis of the disease. *Digestion* 1993; **54**: 148-155 [PMID: 8359556]

6 **Mullady DK**, Yadav D, Amann ST, O'Connell MR, Barmada MM, Elta GH, Scheiman JM, Wamsteker EJ, Chey WD, Korneffel ML, Weinman BM, Slivka A, Sherman S, Hawes RH, Brand RE, Burton FR, Lewis MD, Gardner TB, Gelrud A, DiSario J, Baillie J, Banks PA, Whitcomb DC, Anderson MA. Type of pain, pain-associated complications, quality of life, disability and resource utilisation in chronic pancreatitis: a prospective cohort study. *Gut* 2011; **60**: 77-84 [PMID: 21148579 DOI: 10.1136/gut.2010.213835]

7 **Amann ST**, Yadav D, Barmada MM, O'Connell M, Kennard ED, Anderson M, Baillie J, Sherman S, Romagnuolo J, Hawes RH, Alkaade S, Brand RE, Lewis MD, Gardner TB, Gelrud A, Money ME, Banks PA, Slivka A, Whitcomb DC. Physical and mental quality of life in chronic pancreatitis: a case-control study from the North American Pancreatitis Study 2 cohort. *Pancreas* 2013; **42**: 293-300 [PMID: 23357924 DOI: 10.1097/MPA.0b013e31826532e7]

8 **Gardner TB**, Kennedy AT, Gelrud A, Banks PA, Vege SS, Gordon SR, Lacy BE. Chronic pancreatitis and its effect on employment and health care experience: results of a prospective American multicenter study. *Pancreas* 2010; **39**: 498-501 [PMID: 20118821 DOI: 10.1097/MPA.0b013e3181c5c693]

9 **Drewes AM**, Krarup AL, Detlefsen S, Malmstrøm ML, Dimcevski G, Funch-Jensen P. Pain in chronic pancreatitis: the role of neuropathic pain mechanisms. *Gut* 2008; **57**: 1616-1627 [PMID: 18566105 DOI: 10.1136/gut.2007.146621]

10 **Woolf CJ**. Pain: moving from symptom control toward mechanism-specific pharmacologic management. *Ann Intern Med* 2004; **140**: 441-451 [PMID: 15023710]

11 **Lieb JG**, Forsmark CE. Review article: pain and chronic pancreatitis. *Aliment Pharmacol Ther* 2009; **29**: 706-719 [PMID: 19284407 DOI: 10.1111/j.1365-2036.2009.03931.x]

12 **Anaparthy R**, Pasricha PJ. Pain and chronic pancreatitis: is it the plumbing or the wiring? *Curr Gastroenterol Rep* 2008; **10**: 101-106 [PMID: 18462594]

13 **White TT**, Bourde J. A new observation on human intraductal pancreatic pressure. *Surg Gynecol Obstet* 1970; **130**: 275-278 [PMID: 4983472]

14 **Sato T**, Miyashita E, Yamauchi H, Matsuno S. The role of surgical treatment for chronic pancreatitis. *Ann Surg* 1986; **203**: 266-271 [PMID: 2420294]

15 **Okazaki K**, Yamamoto Y, Ito K. Endoscopic measurement of papillary sphincter zone and pancreatic main ductal pressure in patients with chronic pancreatitis. *Gastroenterology* 1986; **91**: 409-418 [PMID: 3721126]

16 **Laugier R**. Dynamic endoscopic manometry of the response to secretin in patients with chronic pancreatitis. *Endoscopy* 1994; **26**: 222-227 [PMID: 8026369 DOI: 10.1055/s-2007-1008947]

17 **Novis BH**, Bornman PC, Girdwood AW, Marks IN. Endoscopic manometry of the pancreatic duct and sphincter zone

in patients with chronic pancreatitis. *Dig Dis Sci* 1985; **30**: 225-228 [PMID: 3971834]

18 **Rolny P**, Arlebäck A, Järnerot G, Andersson T. Endoscopic manometry of the sphincter of Oddi and pancreatic duct in chronic pancreatitis. *Scand J Gastroenterol* 1986; **21**: 415-420 [PMID: 3726450]

19 **Ugljesič M**, Bulajič M, Milosavljevič T, Stimec B. Endoscopic manometry of the sphincter of Oddi and pancreatic duct in patients with chronic pancreatitis. *Int J Pancreatol* 1996; **19**: 191-195 [PMID: 8807364 DOI: 10.1007/BF02787367]

20 **Vestergaard H**, Kruse A, Rokkjaer M, Frøbert O, Thommesen P, Funch-Jensen P. Endoscopic manometry of the sphincter of Oddi and the pancreatic and biliary ducts in patients with chronic pancreatitis. *Scand J Gastroenterol* 1994; **29**: 188-192 [PMID: 8171290]

21 **Ebbehøj N**, Svendsen LB, Madsen P. Pancreatic tissue pressure: techniques and pathophysiological aspects. *Scand J Gastroenterol* 1984; **19**: 1066-1068 [PMID: 6533777]

22 **Ebbehøj N**, Borly L, Madsen P, Svendsen LB. Pancreatic tissue pressure and pain in chronic pancreatitis. *Pancreas* 1986; **1**: 556-558 [PMID: 3562446]

23 **Ebbehøj N**, Borly L, Bülow J, Rasmussen SG, Madsen P. Evaluation of pancreatic tissue fluid pressure and pain in chronic pancreatitis. A longitudinal study. *Scand J Gastroenterol* 1990; **25**: 462-466 [PMID: 2359973]

24 **Manes G**, Büchler M, Pieramico O, Di Sebastiano P, Malfertheiner P. Is increased pancreatic pressure related to pain in chronic pancreatitis? *Int J Pancreatol* 1994; **15**: 113-117 [PMID: 8071569]

25 **Patel AG**, Toyama MT, Alvarez C, Nguyen TN, Reber PU, Ashley SW, Reber HA. Pancreatic interstitial pH in human and feline chronic pancreatitis. *Gastroenterology* 1995; **109**: 1639-1645 [PMID: 7557149]

26 **Zhu Y**, Colak T, Shenoy M, Liu L, Pai R, Li C, Mehta K, Pasricha PJ. Nerve growth factor modulates TRPV1 expression and function and mediates pain in chronic pancreatitis. *Gastroenterology* 2011; **141**: 370-377 [PMID: 21473865 DOI: 10.1053/j.gastro.2011.03.046]

27 **Schwartz ES**, La JH, Scheff NN, Davis BM, Albers KM, Gebhart GF. TRPV1 and TRPA1 antagonists prevent the transition of acute to chronic inflammation and pain in chronic pancreatitis. *J Neurosci* 2013; **33**: 5603-5611 [PMID: 23536075 DOI: 10.1523/JNEUROSCI.1806-12.2013]

28 **Warshaw AL**, Banks PA, Fernández-Del Castillo C. AGA technical review: treatment of pain in chronic pancreatitis. *Gastroenterology* 1998; **115**: 765-776 [PMID: 9721175]

29 **Andrén-Sandberg A**, Hoem D, Gislason H. Pain management in chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2002; **14**: 957-970 [PMID: 12352215]

30 **Frøkjær JB**, Olesen SS, Drewes AM. Fibrosis, atrophy, and ductal pathology in chronic pancreatitis are associated with pancreatic function but independent of symptoms. *Pancreas* 2013; **42**: 1182-1187 [PMID: 24048457]

31 **Balci NC**, Perman WH, Saglam S, Akisik F, Fattahi R, Bilgin M. Diffusion-weighted magnetic resonance imaging of the pancreas. *Top Magn Reson Imaging* 2009; **20**: 43-47 [PMID: 19687725 DOI: 10.1097/RMR.0b013e3181b48667]

32 **Treede RD**, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology* 2008; **70**: 1630-1635 [PMID: 18003941 DOI: 10.1212/01.wnl.0000282763.29778.59]

33 **Julius D**, Basbaum AI. Molecular mechanisms of nociception. *Nature* 2001; **413**: 203-210 [PMID: 11557989 DOI: 10.1038/35093019]

34 **Xu GY**, Winston JH, Shenoy M, Yin H, Pendyala S, Pasricha PJ. Transient receptor potential vanilloid 1 mediates hyperalgesia and is up-regulated in rats with chronic pancreatitis. *Gastroenterology* 2007; **133**: 1282-1292 [PMID: 17698068 DOI: 10.1053/j.gastro.2007.06.015]

35 **Hartel M**, di Mola FF, Selvaggi F, Mascetta G, Wente MN, Felix K, Giese NA, Hinz U, Di Sebastiano P, Büchler MW, Friess H. Vanilloids in pancreatic cancer: potential for chemotherapy and pain management. *Gut* 2006; **55**: 519-528 [PMID: 16174661 DOI: 10.1136/gut.2005.073205]

36 **Friess H**, Zhu ZW, di Mola FF, Kulli C, Graber HU, Andren-Sandberg A, Zimmermann A, Korc M, Reinshagen M, Büchler MW. Nerve growth factor and its high-affinity receptor in chronic pancreatitis. *Ann Surg* 1999; **230**: 615-624 [PMID: 10561084]

37 **Zhu ZW**, Friess H, Wang L, Zimmermann A, Büchler MW. Brain-derived neurotrophic factor (BDNF) is upregulated and associated with pain in chronic pancreatitis. *Dig Dis Sci* 2001; **46**: 1633-1639 [PMID: 11508661]

38 **Gebhart GF**. Visceral pain-peripheral sensitisation. *Gut* 2000; **47** Suppl 4: iv54-iv5; discussion iv58 [PMID: 11076915]

39 **Anand P**, Aziz Q, Willert R, van Oudenhove L. Peripheral and central mechanisms of visceral sensitization in man. *Neurogastroenterol Motil* 2007; **19**: 29-46 [PMID: 17280584 DOI: 10.1111/j.1365-2982.2006.00873.x]

40 **Woolf CJ**, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science* 2000; **288**: 1765-1769 [PMID: 10846153]

41 **Bockman DE**, Buchler M, Malfertheiner P, Beger HG. Analysis of nerves in chronic pancreatitis. *Gastroenterology* 1988; **94**: 1459-1469 [PMID: 3360267]

42 **Keith RG**, Keshavjee SH, Kerenyi NR. Neuropathology of chronic pancreatitis in humans. *Can J Surg* 1985; **28**: 207-211 [PMID: 3995416]

43 **Ceyhan GO**, Demir IE, Rauch U, Bergmann F, Müller MW, Büchler MW, Friess H, Schäfer KH. Pancreatic neuropathy results in "neural remodeling" and altered pancreatic innervation in chronic pancreatitis and pancreatic cancer. *Am J Gastroenterol* 2009; **104**: 2555-2565 [PMID: 19568227 DOI: 10.1038/ajg.2009.380]

44 **Ceyhan GO**, Bergmann F, Kadihasanoglu M, Altintas B, Demir IE, Hinz U, Müller MW, Giese T, Büchler MW, Giese NA, Friess H. Pancreatic neuropathy and neuropathic pain--a comprehensive pathomorphological study of 546 cases. *Gastroenterology* 2009; **136**: 177-186.e1 [PMID: 18992743 DOI: 10.1053/j.gastro.2008.09.029]

45 **Woolf CJ**. Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 2011; **152**: S2-15 [PMID: 20961685 DOI: 10.1016/j.pain.2010.09.030]

46 **Kuner R**. Central mechanisms of pathological pain. *Nat Med* 2010; **16**: 1258-1266 [PMID: 20948531 DOI: 10.1038/nm.2231]

47 **Latremoliere A**, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J Pain* 2009; **10**: 895-926 [PMID: 19712899 DOI: 10.1016/j.jpain.2009.06.012]

48 **Arendt-Nielsen L**, Laursen RJ, Drewes AM. Referred pain as an indicator for neural plasticity. *Prog Brain Res* 2000; **129**: 343-356 [PMID: 11098702]

49 **Drewes AM**, Pedersen J, Liu W, Arendt-Nielsen L, Gregersen H. Controlled mechanical distension of the human oesophagus: sensory and biomechanical findings. *Scand J Gastroenterol* 2003; **38**: 27-35 [PMID: 12608461]

50 **Dimcevski G**, Sami SA, Funch-Jensen P, Le Pera D, Valeriani M, Arendt-Nielsen L, Drewes AM. Pain in chronic pancreatitis: the role of reorganization in the central nervous system. *Gastroenterology* 2007; **132**: 1546-1556 [PMID: 17408654 DOI: 10.1053/j.gastro.2007.01.037]

51 **Buscher HC**, Wilder-Smith OH, van Goor H. Chronic pancreatitis patients show hyperalgesia of central origin: a pilot study. *Eur J Pain* 2006; **10**: 363-370 [PMID: 16087373 DOI: 10.1016/j.ejpain.2005.06.006]

52 **Olesen SS**, Brock C, Krarup AL, Funch-Jensen P, Arendt-Nielsen L, Wilder-Smith OH, Drewes AM. Descending

inhibitory pain modulation is impaired in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2010; **8**: 724-730 [PMID: 20304100 DOI: 10.1016/j.cgh.2010.03.005]

53 **Olesen SS**, Frøkjær JB, Lelic D, Valeriani M, Drewes AM. Pain-associated adaptive cortical reorganisation in chronic pancreatitis. *Pancreatology* 2010; **10**: 742-751 [PMID: 21273802 DOI: 10.1159/000321644]

54 **Olesen SS**, Hansen TM, Graversen C, Steimle K, Wilder-Smith OH, Drewes AM. Slowed EEG rhythmicity in patients with chronic pancreatitis: evidence of abnormal cerebral pain processing? *Eur J Gastroenterol Hepatol* 2011; **23**: 418-424 [PMID: 21399506 DOI: 10.1097/MEG.0b013e3283457b09]

55 **Olesen SS**, Hansen TM, Graversen C, Valeriani M, Drewes AM. Cerebral excitability is abnormal in patients with painful chronic pancreatitis. *Eur J Pain* 2013; **17**: 46-54 [PMID: 22508470 DOI: 10.1002/j.1532-2149.2012.00155.x]

56 **Frøkjær JB**, Andersen LW, Brock C, Simrén M, Ljungberg M, Søfteland E, Dimcevski G, Yavarian Y, Gregersen H, Drewes AM. Altered brain microstructure assessed by diffusion tensor imaging in patients with diabetes and gastrointestinal symptoms. *Diabetes Care* 2013; **36**: 662-668 [PMID: 23139372 DOI: 10.2337/dc12-1131]

57 **Frøkjær JB**, Bouwense SA, Olesen SS, Lundager FH, Eskildsen SF, van Goor H, Wilder-Smith OH, Drewes AM. Reduced cortical thickness of brain areas involved in pain processing in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2012; **10**: 434-8.e1 [PMID: 22155560 DOI: 10.1016/j.cgh.2011.11.024]

58 **Bouwense SA**, Olesen SS, Drewes AM, Frøkjær JB, van Goor H, Wilder-Smith OH. Is altered central pain processing related to disease stage in chronic pancreatitis patients with pain? An exploratory study. *PLoS One* 2013; **8**: e55460 [PMID: 23405154 DOI: 10.1371/journal.pone.0055460]

59 **Vera-Portocarrero LP**, Xie JY, Kowal J, Ossipov MH, King T, Porreca F. Descending facilitation from the rostral ventromedial medulla maintains visceral pain in rats with experimental pancreatitis. *Gastroenterology* 2006; **130**: 2155-2164 [PMID: 16762636 DOI: 10.1053/j.gastro.2006.03.025]

60 **Bouwense SA**, Buscher HC, van Goor H, Wilder-Smith OH. Has central sensitization become independent of nociceptive input in chronic pancreatitis patients who fail thoracoscopic splanchnicectomy? *Reg Anesth Pain Med* 2011; **36**: 531-536 [PMID: 22005656 DOI: 10.1097/AAP.0b013e31822e0d4a]

61 **Boerma D**, Obertop H, Gouma DJ. Pancreatic pseudocysts in chronic pancreatitis. Surgical or interventional drainage? *Ann Ital Chir* 2000; **71**: 43-50 [PMID: 10829523]

62 **Andrén-Sandberg A**, Dervenis C. Pancreatic pseudocysts in the 21st century. Part I: classification, pathophysiology, anatomic considerations and treatment. *JOP* 2004; **5**: 8-24 [PMID: 14730118]

63 **Ammann RW**, Akovbiantz A, Largiader F, Schueler G. Course and outcome of chronic pancreatitis. Longitudinal study of a mixed medical-surgical series of 245 patients. *Gastroenterology* 1984; **86**: 820-828 [PMID: 6706066]

64 **Aghdassi A**, Mayerle J, Kraft M, Sielenkämper AW, Heidecke CD, Lerch MM. Diagnosis and treatment of pancreatic pseudocysts in chronic pancreatitis. *Pancreas* 2008; **36**: 105-112 [PMID: 18376299 DOI: 10.1097/MPA.0b013e31815a8887]

65 **Gouyon B**, Lévy P, Ruszniewski P, Zins M, Hammel P, Vilgrain V, Sauvanet A, Belghiti J, Bernades P. Predictive factors in the outcome of pseudocysts complicating alcoholic chronic pancreatitis. *Gut* 1997; **41**: 821-825 [PMID: 9462217]

66 **Vijungco JD**, Prinz RA. Management of biliary and duodenal complications of chronic pancreatitis. *World J Surg* 2003; **27**: 1258-1270 [PMID: 14534824 DOI: 10.1007/s00268-003-7246-7]

67 **Abdallah AA**, Krige JE, Bornman PC. Biliary tract obstruction in chronic pancreatitis. *HPB (Oxford)* 2007; **9**: 421-428 [PMID: 18345288 DOI: 10.1080/13651820701774883]

68 **Kalvaria I**, Bornman PC, Marks IN, Girdwood AH, Bank L, Kottler RE. The spectrum and natural history of common bile duct stenosis in chronic alcohol-induced pancreatitis. *Ann Surg* 1989; **210**: 608-613 [PMID: 2818030]

69 **Prinz RA**, Aranha GV, Greenlee HB. Combined pancreatic duct and upper gastrointestinal and biliary tract drainage in chronic pancreatitis. *Arch Surg* 1985; **120**: 361-366 [PMID: 3970672]

70 **Kahl S**, Zimmermann S, Genz I, Schmidt U, Pross M, Schulz HU, Malfertheiner P. Biliary strictures are not the cause of pain in patients with chronic pancreatitis. *Pancreas* 2004; **28**: 387-390 [PMID: 15097855]

71 **Chebli JM**, de Souza AF, Gaburri PD, Bastos KV, Ribeiro TC, Filho RJ, Chebli LA, Castro Ferreira LE. Prevalence and pathogenesis of duodenal ulcer in chronic alcoholic pancreatitis. *J Clin Gastroenterol* 2002; **35**: 71-74 [PMID: 12080230]

72 **Schulze S**, Thorsgaard Pedersen N, Jørgensen MJ, Møllmann KM, Rune SJ. Association between duodenal bulb ulceration and reduced exocrine pancreatic function. *Gut* 1983; **24**: 781-783 [PMID: 6884816]

73 **Saunders JH**, Cargill JM, Wormsley KG. Gastric secretion of acid in patients with pancreatic disease. *Digestion* 1978; **17**: 365-369 [PMID: 669079]

74 **Piubello W**, Vantini I, Scuro LA, Novelli P, Benini L, Brocco G, Cavallini G. Gastric secretion, gastroduodenal histological changes, and serum gastrin in chronic alcoholic pancreatitis. *Am J Gastroenterol* 1982; **77**: 105-110 [PMID: 7072677]

75 **Weber SM**, Rikkers LF. Splenic vein thrombosis and gastrointestinal bleeding in chronic pancreatitis. *World J Surg* 2003; **27**: 1271-1274 [PMID: 14502405 DOI: 10.1007/s00268-003-7247-6]

76 **ROSCH J**, HERFORT K. Contribution of splenoportography to the diagnosis of diseases of the pancreas. II. Inflammatory diseases. *Acta Med Scand* 1962; **171**: 263-272 [PMID: 14493804]

77 **Rignault D**, Mine J, Moine D. Splenoportographic changes in chronic pancreatitis. *Surgery* 1968; **63**: 571-575 [PMID: 5689513]

78 **Bernades P**, Baetz A, Lévy P, Belghiti J, Menu Y, Fékété F. Splenic and portal venous obstruction in chronic pancreatitis. A prospective longitudinal study of a medical-surgical series of 266 patients. *Dig Dis Sci* 1992; **37**: 340-346 [PMID: 1735356]

79 **Brock C**, Olesen SS, Olesen AE, Frøkjaer JB, Andresen T, Drewes AM. Opioid-induced bowel dysfunction: pathophysiology and management. *Drugs* 2012; **72**: 1847-1865 [PMID: 22950533 DOI: 10.2165/11634970-000000000-00000]

80 **Tuteja AK**, Biskupiak J, Stoddard GJ, Lipman AG. Opioid-induced bowel disorders and narcotic bowel syndrome in patients with chronic non-cancer pain. *Neurogastroenterol Motil* 2010; **22**: 424-30, e96 [PMID: 20100280 DOI: 10.1111/j.1365-2982.2009.01458.x]

81 **Casellas F**, Guarner L, Vaquero E, Antolín M, de Gracia X, Malagelada JR. Hydrogen breath test with glucose in exocrine pancreatic insufficiency. *Pancreas* 1998; **16**: 481-486 [PMID: 9598808]

82 **Garcés MC**, Gómez-Cerezo J, Alba D, Codoceo R, Vázquez-Muñoz E, Arnalich F, Barbado FJ, Vázquez JJ. Relationship of basal and postprandial intraduodenal bile acid concentrations and plasma cholecystokinin levels with abdominal pain in patients with chronic pancreatitis. *Pancreas* 1998; **17**: 397-401 [PMID: 9821182]

83 **Xie JY**, Herman DS, Stiller CO, Gardell LR, Ossipov MH, Lai J, Porreca F, Vanderah TW. Cholecystokinin in the rostral ventromedial medulla mediates opioid-induced hyperalgesia and antinociceptive tolerance. *J Neurosci* 2005; **25**: 409-416 [PMID: 15647484 DOI: 10.1523/JNEUROSCI.4054-04.2005]

84 **Shiratori K**, Takeuchi T, Satake K, Matsuno S. Clinical eval-

uation of oral administration of a cholecystokinin-A receptor antagonist (loxiglumide) to patients with acute, painful attacks of chronic pancreatitis: a multicenter dose-response study in Japan. *Pancreas* 2002; **25**: e1-e5 [PMID: 12131781]

85 **Buscher HC**, van Goor H, Sweep CG, Lenders JW, Wilder-Smith OH. Increased sympathetic activity in chronic pancreatitis patients is associated with hyperalgesia. *J Pain Palliat Care Pharmacother* 2010; **24**: 362-366 [PMID: 21133744 DOI: 10.3109/15360288.2010.519762]

86 **Chandrasekharan B**, Srinivasan S. Diabetes and the enteric nervous system. *Neurogastroenterol Motil* 2007; **19**: 951-960 [PMID: 17971027 DOI: 10.1111/j.1365-2982.2007.01023.x]

87 **Demir IE**, Tieftrunk E, Maak M, Friess H, Ceyhan GO. Pain mechanisms in chronic pancreatitis: of a master and his fire. *Langenbecks Arch Surg* 2011; **396**: 151-160 [PMID: 21153480 DOI: 10.1007/s00423-010-0731-1]

P- Reviewers: Farré A, Frulloni L, Seicean A **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7292

World J Gastroenterol 2013 November 14; 19(42): 7292-7301
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Pharmacological pain management in chronic pancreatitis

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Received: June 8, 2013 **Revised:** August 5, 2013
Accepted: August 20, 2013
Published online: November 14, 2013

Abstract

Intense abdominal pain is a prominent feature of chronic pancreatitis and its treatment remains a major clinical challenge. Basic studies of pancreatic nerves and experimental human pain research have provided evidence that pain processing is abnormal in these patients and in many cases resembles that seen in neuropathic and chronic pain disorders. An important ultimate outcome of such aberrant pain processing is that once the disease has advanced and the pathophysiological processes are firmly established, the generation of pain can become self-perpetuating and independent of the initial peripheral nociceptive drive. Consequently, the management of pain by traditional methods based on nociceptive deafferentation (*e.g.*, surgery and visceral nerve blockade) becomes difficult and often ineffective. This novel and improved understanding of pain aetiology requires a paradigm shift in pain management of chronic pancreatitis. Modern mechanism based pain treatments taking into account altered pain processing are likely to increasingly replace invasive therapies targeting the nociceptive source, which should be reserved for special and carefully selected cases. In this review, we offer an overview of the current available pharmacological options for pain management in chronic pancreatitis. In addition, future options for pain management are discussed with special emphasis on personalized pain medicine and multidisciplinarity.

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Key words: Chronic pancreatitis; Pain; Treatment; Pharmacology; Analgesics; Adjuvant analgesics

Core tip: Pharmacological pain management in chronic pancreatitis is complicated and requires a multidisciplinary approach. Identification of risk factors associated with disease progression and evaluation of extra pancreatic causes of pain and complications is essential in all patients. Analgesics are typically titrated according to the World Health Organization ladder principle, but in some situations a top-down approach may be useful to control pain and avoid sensitization of central pain pathways. Adjuvant analgesics and combinations of drugs should be considered at an early stage. Non-encapsulated enzyme therapy, somastotatin-analogues and antioxidants can be considered as supplements to conventional analgesics in special situations.

Olesen SS, Juel J, Graversen C, Kolesnikov Y, Wilder-Smith

OHG, Drewes AM. Pharmacological pain management in chronic pancreatitis. *World J Gastroenterol* 2013; 19(42): 7292-7301 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7292.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7292

INTRODUCTION

Chronic pancreatitis (CP) remains a major source of morbidity in the Northern Europe, with an annual incidence of approximately 10/100000 inhabitants[1]. It is a disease characterised by progressive destruction of the pancreatic gland and is typically characterised by severe abdominal pain. As the disease evolves, significant impairment of exocrine as well as endocrine functions also become evident[2,3]. The aetiological risk-factors associated with CP are multiple and involve both genetic and environmental factors. In the Northern Europe, excessive alcohol consumption is the leading cause of CP, although genetic susceptibility is also recognised as playing an increasing role[1,4].

From the perspective of the patients (and their doctors) pain is the most significant symptom in CP, and most patients develop chronic pain during the course of their disease. The classic description of the pain is that of a constant, severe, dull ache in the mid-epigastrium, which often radiates to the back. It is typically worsened by high-fat foods, and pain attacks may last for days. However, just as the disease has different causes and morphological expressions, this classic pain pattern is not universal, and the location, character and quality of pain can be quite variable[2]. Furthermore, pain has been associated with malnutrition, narcotic addiction, physical and emotional disability, and major socioeconomic problems. Consequently, the clinical evaluation of pain is often blurred by addiction to alcohol and narcotic analgesics as well as by the personality disorders underlying these dependencies[3,5]. In view of this complex clinical presentation, it is not surprising that treatment of pain in patients with CP is challenging and often unsuccessful[6].

The aim of this review is to summarise current available pharmacological therapies for pain in CP. In addition, future options for pain management are discussed with special emphasis on personalized pain medicine and multidisciplinarity.

OVERVIEW OF PAIN MECHANISMS IN CP

A detailed overview of the complex pain mechanisms underlying pain in CP is beyond the scope of this review and provided elsewhere in this issue of the journal. It is important to emphasise that none of the current acknowledged theories are mutually exclusive, and it is most likely that several pain mechanisms act in concert to cause pain in the individual patient.

Historically, the focus of pain treatment has been on the pancreatic gland as a nociceptive source, based on the assumption that pain is generated by local pathology within or in close proximity to the pancreas. This mechanistic understanding of pain, has for many years, been the most widely accepted theory regarding the origin of pain in CP[7]. However, there is no direct relationship between the presence of pancreatic pathology such as duct dilation, pancreatic duct stones, pancreatic duct strictures, etc. and abdominal pain in CP pain patients[8-11]. Furthermore, the experimental evidence supporting this theory is sparse and findings have been conflicting[12].

On the contrary, many current theories of the pathophysiology of CP postulate that in a high number of cases, repeated episodes of inflammation and pancreatic injury drive the process within the gland towards irreversible injury and are associated with damage to the pancreatic nerves[7,13,14]. Key in this theory is the recognition that the resulting ongoing and aggressive nociceptive input is likely associated with altered function of the pain processing system, particularly at the central level[15-18]. An important ultimate outcome of such aberrant pain processing is that once the disease has advanced and the pathophysiological processes are firmly established, the generation of pain can become self-perpetuating and independent of the initial peripheral nociceptive drive[19,20]. Consequently, the management of pain by traditional methods based on nociceptive deafferentation (*e.g.*, surgery and visceral nerve blockade) becomes difficult and ineffective[20]. This novel and improved understanding of pain aetiology requires a paradigm shift in pain management of CP. Hence, modern mechanism based pain treatments taking into account altered pain processing are likely to increasingly replace invasive therapies targeting the nociceptive source, which should be reserved for special and carefully selected cases.

RISK-FACTOR MODIFICATION AND PROPHYLAXIS

The risk-factors associated with CP can be classified according to the MANNHEIM risk-factor classification system[4]. In this system, the multiple (M) risk factors underlying CP are categorised into six major subcategories of alcohol consumption (A), nicotine consumption (N), nutritional factors (N), hereditary factors (H), efferent pancreatic duct factors (E), immunological factors (I), and various rare miscellaneous and metabolic (M) factors. The rationale for modifying these risk-factors is to reduce recurrent injury to the pancreas. Hence, with repeated episodes of acute inflammation triggered by one or more risk factors, the inflammatory environment within the pancreas shifts towards chronic inflammation, with subsequent activation of pancreatic stellate cells, fibrinogenesis, and irreversible pancreatic damage[21]. Although not well established for all risk-factors (see below), it seems likely that prevention of recurrent pancreatitis attacks, clinical or sub-clinical, by risk-factor modification, will translate into a slowing of disease progression, less exo-

Table 1 Recommended risk factor modifications in chronic pancreatitis according to the MANNHEIM criteria

Risk factor	Treatment	Comments
Alcohol	Alcohol cessation	Decrease disease progression and may have beneficial effects on pain
Nicotine	Smoking cessation	Decrease disease progression and may have beneficial effects on pain
Nutritional	No specific recommendations	No prospective data
Hereditary	Endoscopic surveillance	Currently no formal evidence, a prospective trial has been initiated
	Pancreatectomy with autolog stem cell transplantation	Preferred strategy in some United States centers
Efferent duct	Endoscopy or surgical interventions	The benefit of intervention is controversial
Immunological	Steroid treatment	Treatment of autoimmune pancreatitis follows guidelines provided in *e.g.*, Ref. 32
Metabolic	Lipid lowering therapy, parathyroidectomy, *etc.*	Consider referral to an endocrinologist

Table 2 Treatment of extrapancreatic causes of pain in chronic pancreatitis

	Treatment	Comments
Peptic ulcer	Proton pump inhibitor +/- eradication of *H. pylori*	Avoid NSAIDs in CP Patients
Pseudocysts	Endoscopic drainage, transcutaneous drainage or surgical drainage	Preferred treatment dependent on pseudocyst localization and morphology
Duodenal obstruction	Endoscopic dilation or surgical therapy	Endoscopic dilation preferred as first line therapy
Bile duct obstruction	Covered metal stent or plastic stent	Controversial, one study found no relationship between bile duct obstruction and pain

NSAID: Nonsteroidal anti-inflammatory drugs; CP: Chronic pancreatitis; *H. pylori*: *Helicobacter pylori*.

crine and endocrine insufficiency and most importantly decreased abdominal pain. In Table 1 recommended risk factor modifications are summarized.

In patients with an alcoholic aetiology of CP, there is evidence to support that cessation of alcohol may have beneficial effects on disease progression and pain[22,23]. Furthermore, there is increasing evidence that tobacco use is also an important and independent risk factor for CP and that cigarette smoking accelerates progression of alcoholic CP[24,25]. Hence, tobacco cessation is highly recommended in these patients, although the association with pain relief has yet to be determined.

Data on the association between nutritional factors and CP are sparse. The consumption of a diet rich in fat and protein was associated with the development of CP in a case-control study[26]. However, retrospective descriptions of daily nutritional habits are difficult and such data may be subject to recall bias. Thus, it is difficult to provide a simple description of past daily nutrition in the majority of patients with CP and these findings needs to be confirmed in a prospective trial before specific recommendations can be made.

In patients with CP following gallstone pancreatitis, prevention of recurrent choledocholithiasis is crucial and reduces further damage to the pancreas[27]. In this situation cholecystectomy is recommended for patients suitable for surgery[28]. Also, patients with recurrent pancreatitis and efferent duct abnormalities such as pancreas divisum may benefit from endoscopic therapy or surgery to decrease the risk of recurrent pancreatitis and progression to CP[29]. However, data on this subject are limited and the optimal treatment of this specific entity is still a subject of controversy.

No specific treatment exists to modify the disease progression in hereditary CP. These patients have a significantly increased risk of pancreatic cancer and surveillance or even total pancreatectomy with autologous islet-cell transplantation is recommended in some centers[30]. Patients with autoimmune pancreatitis comprise a special subset of patients with a potentially curable form of pancreatitis. Management of these patients is beyond the scope of this review and the reader is referred to reference[31].

In CP due to metabolic abnormalities such as hypertriglyceridaemia, maintenance of triglycerides within the normal range would be expected to reduce the chance of repeated pancreatitis attacks and thus progression to CP[32]. Also, patients with hypercalcaemia induced pancreatitis due to hyperparathyroidism should be managed appropriately and - if necessary - referred to an endocrinologist.

TREATMENT OF EXTRA-PANCREATIC CAUSES OF PAIN

In addition to risk factor modifications, extra-pancreatic causes of pain should be thoroughly investigated and treated (Table 2). Peptic ulcers are reported to have an increased prevalence in CP. This is possibly explained by changes in blood flow to the mucosa following attacks of acute pancreatitis as well as deterioration of pancreatic exocrine function resulting in a reduction of bicarbonate concentration and hence acidification of the milieu in the duodenal lumen. Also, increased gastric acid secretion and an increased prevalence of *Helicobacter pylori* in CP have been associated with the increased prevalence of peptic ulcers[33]. Another important source of pain in CP is pseudocysts, which should be investigated by an appropriate radiological work-up and treated accordingly[34]. Some patients may have pain as a consequence of obstruction of adjacent viscera (duodenum or common

Table 3 Current available pharmacological treatments for pain in chronic pancreatitis

Pain mechanism	Treatment option(s)	Comments	Ref.
Raised levels of CCK	Pancreatic enzyme replacement therapy	Only non-enteric coated enzymes have proven effective	[57-65]
	Somatostatin-analogues	Conflicting results, prolonged release formulations may be of value	[67,68]
Pancreatic inflammation and oxidative stress	Antioxidants	Conflicting results, probably most valuable in tropical calcifying CP	[71,72]
Central sensitisation	Antidepressants (TCA, SSRI, SNRI)	Expert opinion, no clinical data (Ref.)	[2]
	Gabapentinoids (Gabapentin/Pregabalin)	Modest effect on pain in a randomised placebo controlled trial (Pregabalin)	[42]
	Ketamine	Reverses hyperalgesia in an experimental pain study	[54]
Analgesics	Tramadol *vs* morphine	No difference in pain relief in a randomised controlled trial, fewer side effects on tramadol treatment	[35]
	Fentanyl *vs* Morphine	No difference in pain relief in a randomised controlled trial	[41]
	Oxycodone *vs* Morphine	Oxycodone superior to morphine on experimental pain measures	[39]
	ADL 10-0101:KOR agonist	KOR agonist superior to morphine on experimental and clinical pain measures. Limited number of patients (n = 6)	[40]

CCK: Cholecystokinin; CP: Chronic pancreatitis; TCA: Tricyclic antidepressant; SSRI: Selective serotonin reuptake inhibitor; SNRI: Serotonin norepinephrine reuptake inhibitor; KOR: Kappa opioid receptor.

bile duct)[35]. However, the mechanisms underlying such "obstructive pain" remain unclear and in the case of bile duct obstruction there is evidence to the contrary[36].

ANALGESICS

The standard guideline for analgesic therapy in CP patients follows the principles of the "pain relief ladder" provided by the World Health Organization (WHO)[37]. This principle is based on the serial introduction of drugs with increasing analgesic potency, titrated until pain relief is obtained. However, in patients with a severe and debilitating pain pattern, a more aggressive approach using opioids combined with adjuvant analgesics as first line therapy (*i.e.,* a top-down approach), is useful to control pain and prevent sensitization of central pain pathways. An overview of the current available pharmacological therapies used to treat pain in CP is reported in Table 3.

Paracetamol is usually the preferred drug in level I analgesia due to its limited side effects. It has analgesic and antipyretic activity that work through central and peripheral non-opioid mechanisms, which have not yet been fully characterised[38]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are particular useful for treating musculoskeletal pain and are in general less favourable for visceral pain because of their toxicity to the GI tract[39]. Consequently, we recommend avoiding NSAIDs for painful CP.

Codeine is a weak opioid in level Ⅱ analgesia, but is still associated with the same spectrum of opioid-related side effects seen for stronger opioids, *e.g.*, constipation, nausea, dyspepsia amongst other symptoms involved in opioid-induced bowel dysfunction[40]. Tramadol possesses both a weak opioid agonist activity along with an effect on noradrenaline and serotonin uptake in the spinal cord. It has been shown to be more potent than codeine and may be considered as a halfway house between level Ⅱ and level Ⅲ analgesics. Tramadol was also shown to be more efficacious than morphine in patients with CP, with fewer gastrointestinal side effects for the same level of analgesia[41].

Strong opioids, such as morphine, mainly exert their analgesic effects in the central nervous system, although it is now well known that opioid receptors are synthesised in the dorsal root ganglia and transported towards both central and peripheral nerve terminals[42]. Several opioid receptors exist, including the μ-receptor, δ-receptor and the κ-receptor[43]. Most clinically available opioids have their primary activity at the μ-receptor and have been used widely to treat pain in CP patients[6]. However, animal studies have suggested that activation of the κ-receptor may be more efficacious for attenuation of gastrointestinal pain[44]. In keeping with these findings, oxycodone (an opioid targeting the μ-, δ- and κ-receptor) was shown to attenuate experimental visceral pain better than morphine in CP patients[45]. Also, in a pilot study including six CP patients with chronic abdominal pain, infusion of a peripherally restricted κ-receptor agonist (ADL 10-0101) - but not placebo - reduced clinical and experimental pain scores[46]. These findings were not replicated in patients with pain due to pancreatic cancer[47], but this may relate to the confounders associated with clinical studies on opioids[48]. Taken together, these findings may suggest differentiated effects of opioids for pain management in CP patients. However, it must be emphasized that data from well-designed clinical studies with long-term follow-up are not yet available.

Opioids used in the outpatient clinic can be administered either orally (*i.e.,* tablets) or transdermally (*i.e.,* patch formulation). In an open label randomized crossover trial, transdermal fentanyl plaster was compared to sustained release morphine tablets in an equipotent dosage regime[49]. No significant differences were found for pain control, patients' preference or quality of life, while 44% of patients treated with fentanyl plaster reported skin side effects. Taken together with the increased costs of patch formulation, the authors concluded that transdermal administration of opioids cannot be recommended as first line opioid therapy for CP, but should be reserved to patients having trouble with tablet ingestion[49].

As discussed above, CP patients may be suffering from hyperalgesia due to sensitization of the central nervous system[14,19]. In general, opioids are not very effective in treating established central sensitization and may even cause hyperalgesia themselves (*i.e.,* opioid induced hyperalgesia)[50]. Furthermore, opioid induced bowel dysfunction is a common problem in clinical practice and typically manifests as abdominal discomfort or even diffuses abdominal pain[40]. Taken together, opioid based therapies often become ineffective and associated with gastrointestinal side effects in the context of advanced CP and hence other treatments are highly warranted.

Adjuvant analgesics

Adjuvant analgesics are a heterogeneous group of drugs initially developed for indications other than pain. However, many have proven effective in painful conditions, which has now been widely recognised as a separate therapeutic indication. Adjuvant analgesics modify the nociceptive processes through several modes of action, including anxiolytic effects (benzodiazepines, alpha-2-delta ligands), antidepressive effects (antidepressants), and anti-hyperalgesic effects (antidepressants, alpha-2-delta ligands). Although they have been widely used to treat pain associated with CP, only the alpha-2-delta ligand pregabalin has been studied in the context of painful CP[2,51]. Hence, in a placebo controlled double blinded randomized trial, we recently demonstrated the efficacy of pregabalin as an adjuvant analgesic for pain in CP. We found that CP patients treated with pregabalin escalated to a maximal dose of 600 mg *bid* had a significant reduction in self-reported pain scores compared to placebo. Furthermore, the percentage of patients with much or very much improved health status score was higher in the pregabalin group compared to the placebo group. The side effects were relatively few and of mild to moderate severity; with a "drunk feeling" being the most prevalent side effect (35% of patients) and typically showing a ceiling effect after one or two weeks of treatment[51].

The analgesic mechanisms of action underlying pregabalin analgesia are not completely understood, and it probably exerts a range of effects on pain transmission[52,53]. *In vitro* studies indicate that pregabalin binds selectively to the alpha-2-delta subunit of voltage-dependent calcium channels, thereby blocking the influx of calcium into pre-synaptic nerve terminals. This reduces release of excitatory neurotransmitters, including glutamate, noradrenalin and substance P, and dampens pain transmission[54,55]. These findings translate well to experimental pain studies in CP, where antinociceptive effects of pregabalin on electrical evoked pain from the gut and skin were observed, compatible with a reduction of central sensitization[56,57].

Ketamine

Introduced in 1965 as an anesthetic, today ketamine is used not only for anesthesia, but also as a potent analgesic in acute and chronic pain as well as an antihyperalgesic used to reduce central sensitization in various chronic pain conditions. It is a noncompetitive *N*-Methyl-*D*-aspartate (NMDA) receptor antagonist, but it also exerts its analgesic effects through other mechanisms including opioid receptor activation[58]. Sensitization of the central nervous system has been documented in several studies of painful CP and is believed to play a prominent role in pain generation in this entity[15,16]. One of the best-characterized mechanisms in the early phase of central sensitization is activation of the NMDA receptors[59]. Multiple studies have consistently produced positive results regarding the use of ketamine in chronic pain patients with central sensitization and hyperalgesia and it thus comprises an interesting remedy to revert reduce central sensitization and its associated hyperalgesia in CP[60]. This was supported by a double-blinded crossover trial designed to evaluate the effect of ketamine infusion on hyperalgesia associated with CP[61]. Infusion of ketamine temporarily reversed pressure pain hyperalgesia and the underlying sensitized state of the pain system. However, only short-term effects were evaluated and no effect was seen on clinical endpoints. Hence, the use of ketamine for pain in CP is still in its infancy and prospective clinical trials are warranted to establish its role in the management of painful CP.

SUPPLEMENTARY THERAPIES

Pancreatic enzyme therapy

Pancreatic enzyme therapy for pain control in CP has been the subject of several randomized trials and meta-analyses (Table 3). The proposed mechanism of action is the ability to degrade cholecystokinin (CCK) releasing factor in the duodenum and thereby lower CCK[2]. An elevated level of CCK have been reported in CP patients and may generate pain by increasing the pressure in the pancreatic duct (CCK-A), but also through direct activation of nociceptive pathways in the central nervous system (CCK-B)[62,63]. Only non-enteric coated formulations have duodenal protease activity and studies using this type of enzymes have documented improvement in pain[64,65]. In contrast, most studies using enteric coated preparations (which are not active in the duodenum and hence cannot degrade CCK-releasing factor) have not shown any improvement on pain measures[66-69]. One study, however, showed pain relief of enteric coated enzymes during acid inhibition, but this study used a measurement of pain that included symptoms of malabsorption (bloating, gas or cramping), rather than more traditional pain measures[70]. A meta-analysis combining all studies found no effect of enzymes on pain relief in CP[71]. Nevertheless, combining the two types of enzyme formulations in a metanalysis is probably not appropriate given the proposed mechanism of action[72].

Somastotatin-analogues

Somastotatin-analogue inhibits pancreatic secretion by blocking CCK and secretin release and also by a direct inhibitory effect on acinar cells[73]. As discussed above, these effects may alleviate pain through reduction of pancreatic ductal pressure and by lowering the central effects of CCK. There are conflicting data about the efficacy of somastotatin-analogues for pain in CP. While early pilot series of octreotide showed an effect on pain control, this effect could not be confirmed in a double-blind cross-over study enrolling 10 CP patients treated with octreotide (100 μg *tid*) or placebo for 3 d[74]. Although pancreatic secretion measured by fecal chymotrypsin was reduced by octreotide, no differences were seen in pain control or analgesic use. This study has been criticized for its relatively short follow-up and limited wash out period (48 h). Also, four patients had evidence of concrements in the pancreatic duct, which may have compromised the effect of octreotide. In a later pilot study, a long-acting version of octreotide (Octreotide LAR) administered once monthly, was compared to conventional subcutaneous octreotide treatment administered three times daily. Although not significant, there was a trend toward improved pain control for octreotide LAR[75]. These results, however, have never been subject to a formal placebo controlled trial and the role of octreotide treatment for painful CP has so far not been satisfactorily documented. Taken together with the numerous side effects and their cost, a general use of somastotatin-analogues for pain in CP cannot be recommended[76].

Antioxidants

The use of antioxidants for pain control in CP was presented two decades ago, but never gained widely clinical popularity. The proposed analgesic mechanism of action underlying this therapy is an anti-inflammatory and blocking effect on free radicals[77]. Propelled by an Indian randomized placebo controlled trial, antioxidant therapy recently had a rebirth for pain management in CP. In this trial, six months antioxidant therapy was associated with significant and prolonged pain relief compared to placebo[78]. However, these findings were not reproduced by a subsequent study from North America[79]. A possible explanation for this dichotomy may be that the patients included in the two trials were different. While the Indian study mostly included patients with trophic calcifying pancreatitis and malnutrition (and hence deficiency in antioxidants), the American study included a more elderly population who had alcohol as the leading etiology of CP and a normal nutritional condition. Hence, the efficacy of antioxidant therapy may be related to the etiology of CP and its associated malnutrition[80]. This idea was supported by a subgroup analysis of the patients with alcohol etiology in the Indian trial, who, in agreement with the American study, demonstrated no benefit of antioxidants[78]. Taken together, the evidence is not sufficient to recommend antioxidant therapy be used routinely for the typical Western CP patient with alcoholic pancreatitis.

Other treatments

In addition to the abovementioned treatment options, various other pharmacological principles have been used to treat pain associated with CP, including leukotriene antagonism and stimulation with secretin[81,82]. However, none of these treatments have documented any effect on pain and are regarded obsolete by most experts.

INDIVIDUALISED PAIN THERAPY AND FUTURE ANALGESICS FOR PAIN IN CHRONIC PANCREATITIS

A major problem in pain medicine is the lack of knowledge about which treatment suits a specific patient. In a recent study, we tested the ability of quantitative sensory testing to predict the analgesic effect of pregabalin and placebo in patients with CP[83]. Pregabalin effect was associated with pretreatment sensitivity to electric tetanic stimulation of the upper abdominal area (sharing spinal segmental innervation with the pancreatic gland). Hence, patients expressing lower pain thresholds in the "pancreatic viscerotome" were more likely to benefit from pregabalin treatment compared to patients with normal sensitivity[83]. These findings suggest sensitization of spinal neurons in the segment innervated by pancreatic visceral afferents to be an important predictor of pregabalin efficacy in patients with painful CP. Interestingly, this method may be used to tailor pain medication based on patient's individual sensory profile and thus comprises a significant step towards personalized pain medicine.

The novel and improved understanding of pain mechanisms in CP may pave the way for new treatments. Analgesics specifically targeting neural or humoral mediators of pain, such as nerve growth factor (NGF) and transient receptor potential vanilloid-1 antagonists, are currently being tested in clinical trials and hold promise for the future, although these drugs have yet to be tested in patients with CP[84,85]. Recently, a NGF-antagonist (Tanezumab) was shown to relief pain in patients with knee pain due to gonarthrosis[84]. As NGF has been shown to be up-regulated in CP patients and is known to play a pivotal role in the process of peripheral sensitization, NGF-antagonism may be effective for pain relief in CP patients[86].

Multidisciplinary pain treatment

As discussed above the mechanisms underlying pain in CP are highly variable in the individual patient. Consequently, there is no single approach that is effective for all patients and choosing the right algorithm for pain treatment is highly depending of the pathogenesis of pain in the individual situation. Hence, a successful management of pain requires a multidisciplinary approach as illustrated in Figure 1. In addition, establishing a stable doctor-patient relationship is an important factor for a successful treatment outcome[80].

CONCLUSION

Intense abdominal pain is the most prominent feature of CP and its treatment remains a major clinical challenge.

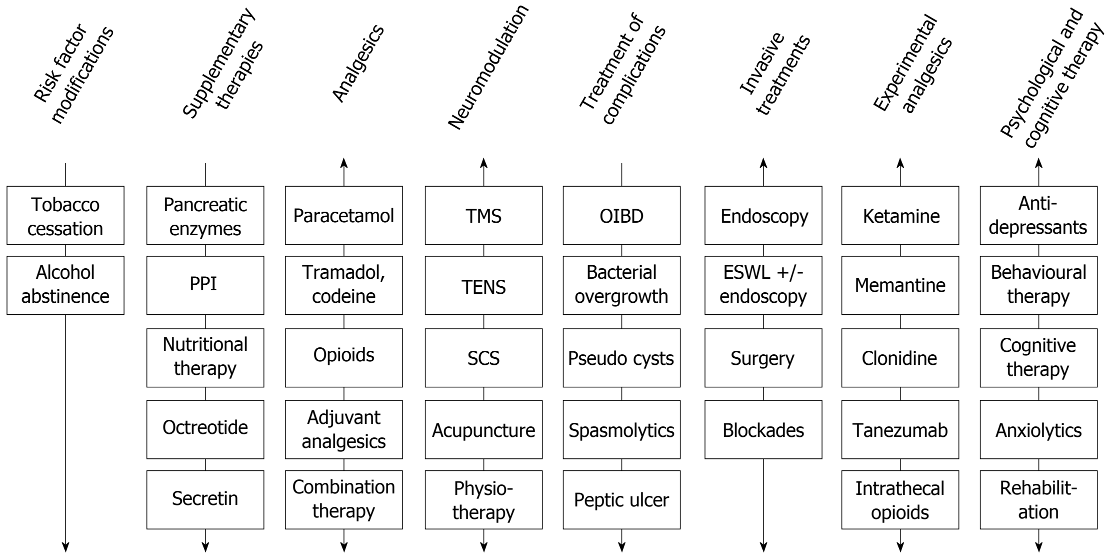

Figure 1 An illustration of the multidisciplinary approach recommended for managing pain in chronic pancreatitis. The mechanisms underlying pain in chronic pancreatitis (CP) are highly variable in the individual patient and there is no single approach that is effective for all patients. Hence, choosing the right algorithm for the pain treatment is highly depending of the pathogenesis of pain in the individual situation. Some treatments follow a typical step-up approach as indicated by the unidirectional arrows. Other treatments follow either a step-up or a top-down approach depending on the specific situation as illustrated by the bidirectional arrows. The latter is seen for example for analgesic therapies, where weak analgesics may be appropriate to control pain in one situation. On the other hand a more aggressive approach, using opioids combined with adjuvant analgesics as first line therapy (*i.e.*, top-down), is useful in patients with a more aggressive and debilitating pain pattern to control pain and prevent sensitization of central pain pathways. Often combination therapies of *e.g.*, opioids or adjuvant analgesics are used. Surgery, endoscopic therapies *etc.* are included in the figure for completeness, although these therapies are beyond the scope of this review. PPI: Proton pump inhibitor; TENS: Transcutaneous electrical nerve stimulation; SCS: Spinal cord stimulation; OIBD: Opioid-induced bowel dysfunction; TMS: Transcranial magnetic stimulation; ESWL: Extracorporeal shock wave lithotripsy.

Medical management requires a multidisciplinary approach including identification of risk factors associated with disease progression and appropriate modification. A systematically evaluation of extra pancreatic causes of pain and complications followed by appropriate treatments is essential in all patients. Analgesics are typically titrated according to the WHO ladder principle, but in some situations a top-down approach may be useful to control pain and avoid sensitization of central pain pathways. Also, adjuvant analgesics should be considered at an early stage and combinations of drugs are often used. Non-encapsulated enzyme therapy, somastotatin-analogues and antioxidants can be considered as supplements to conventional analgesics in special situations. An improved understanding of pain mechanisms in CP will undoubtedly pave the way for new treatments and future strategies should be based on modern mechanism based and personalized pain treatment.

REFERENCES

1 **Andersen BN**, Pedersen NT, Scheel J, Worning H. Incidence of alcoholic chronic pancreatitis in Copenhagen. *Scand J Gastroenterol* 1982; **17**: 247-252 [PMID: 7134849]

2 **Lieb JG**, Forsmark CE. Review article: pain and chronic pancreatitis. *Aliment Pharmacol Ther* 2009; **29**: 706-719 [PMID: 19284407 DOI: 10.1111/j.1365-2036.2009.03931.x]

3 **Andrén-Sandberg A**, Hoem D, Gislason H. Pain management in chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2002; **14**: 957-970 [PMID: 12352215]

4 **Schneider A**, Löhr JM, Singer MV. The M-ANNHEIM classification of chronic pancreatitis: introduction of a unifying classification system based on a review of previous classifications of the disease. *J Gastroenterol* 2007; **42**: 101-119 [PMID: 17351799 DOI: 10.1007/s00535-006-1945-4]

5 **Gardner TB**, Kennedy AT, Gelrud A, Banks PA, Vege SS, Gordon SR, Lacy BE. Chronic pancreatitis and its effect on employment and health care experience: results of a prospective American multicenter study. *Pancreas* 2010; **39**: 498-501 [PMID: 20118821 DOI: 10.1097/MPA.0b013e3181c5c693]

6 **Warshaw AL**, Banks PA, Fernández-Del Castillo C. AGA technical review: treatment of pain in chronic pancreatitis. *Gastroenterology* 1998; **115**: 765-776 [PMID: 9721175]

7 **Anaparthy R**, Pasricha PJ. Pain and chronic pancreatitis: is it the plumbing or the wiring? *Curr Gastroenterol Rep* 2008; **10**: 101-106 [PMID: 18462594]

8 **Bornman PC**, Marks IN, Girdwood AH, Clain JE, Narunsky L, Clain DJ, Wright JP. Is pancreatic duct obstruction or stricture a major cause of pain in calcific pancreatitis? *Br J Surg* 1980; **67**: 425-428 [PMID: 7388340]

9 **Lankisch PG**, Seidensticker F, Löhr-Happe A, Otto J, Creutzfeldt W. The course of pain is the same in alcohol- and nonalcohol-induced chronic pancreatitis. *Pancreas* 1995; **10**: 338-341 [PMID: 7792289]

10 **Jensen AR**, Matzen P, Malchow-Møller A, Christoffersen I. Pattern of pain, duct morphology, and pancreatic function in chronic pancreatitis. A comparative study. *Scand J Gastroenterol* 1984; **19**: 334-338 [PMID: 6740208]

11 **Malfertheiner P**, Büchler M, Stanescu A, Ditschuneit H. Pancreatic morphology and function in relationship to pain in chronic pancreatitis. *Int J Pancreatol* 1987; **2**: 59-66 [PMID: 3681034 DOI: 10.1007/BF02788349]

12 **Fasanella KE**, Davis B, Lyons J, Chen Z, Lee KK, Slivka A, Whitcomb DC. Pain in chronic pancreatitis and pancreatic cancer. *Gastroenterol Clin North Am* 2007; **36**: 335-64, ix [PMID: 17533083 DOI: 10.1016/j.gtc.2007.03.011]

13 **Pasricha PJ**. Unraveling the mystery of pain in chronic pancreatitis. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 140-151 [PMID: 22269952 DOI: 10.1038/nrgastro.2011.274]

14 **Demir IE**, Tieftrunk E, Maak M, Friess H, Ceyhan GO. Pain

mechanisms in chronic pancreatitis: of a master and his fire. *Langenbecks Arch Surg* 2011; **396**: 151-160 [PMID: 21153480 DOI: 10.1007/s00423-010-0731-1]

15 **Olesen SS**, Brock C, Krarup AL, Funch-Jensen P, Arendt-Nielsen L, Wilder-Smith OH, Drewes AM. Descending inhibitory pain modulation is impaired in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2010; **8**: 724-730 [PMID: 20304100 DOI: 10.1016/j.cgh.2010.03.005]

16 **Buscher HC**, Wilder-Smith OH, van Goor H. Chronic pancreatitis patients show hyperalgesia of central origin: a pilot study. *Eur J Pain* 2006; **10**: 363-370 [PMID: 16087373 DOI: 10.1016/j.ejpain.2005.06.006]

17 **Frøkjær JB**, Olesen SS, Gram M, Yavarian Y, Bouwense SA, Wilder-Smith OH, Drewes AM. Altered brain microstructure assessed by diffusion tensor imaging in patients with chronic pancreatitis. *Gut* 2011; **60**: 1554-1562 [PMID: 21610272 DOI: 10.1136/gut.2010.236620]

18 **Frøkjær JB**, Bouwense SA, Olesen SS, Lundager FH, Eskildsen SF, van Goor H, Wilder-Smith OH, Drewes AM. Reduced cortical thickness of brain areas involved in pain processing in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2012; **10**: 434-8.e1 [PMID: 22155560 DOI: 10.1016/j.cgh.2011.11.024]

19 **Bouwense SA**, Olesen SS, Drewes AM, Frøkjær JB, van Goor H, Wilder-Smith OH. Is altered central pain processing related to disease stage in chronic pancreatitis patients with pain? An exploratory study. *PLoS One* 2013; **8**: e55460 [PMID: 23405154 DOI: 10.1371/journal.pone.0055460]

20 **Bouwense SA**, Buscher HC, van Goor H, Wilder-Smith OH. Has central sensitization become independent of nociceptive input in chronic pancreatitis patients who fail thoracoscopic splanchnicectomy? *Reg Anesth Pain Med* 2011; **36**: 531-536 [PMID: 22005656 DOI: 10.1097/AAP.0b013e31822e0d4a]

21 **Witt H**, Apte MV, Keim V, Wilson JS. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology* 2007; **132**: 1557-1573 [PMID: 17466744 DOI: 10.1053/j.gastro.2007.03.001]

22 **Strum WB**. Abstinence in alcoholic chronic pancreatitis. Effect on pain and outcome. *J Clin Gastroenterol* 1995; **20**: 37-41 [PMID: 7884175]

23 **Coté GA**, Yadav D, Slivka A, Hawes RH, Anderson MA, Burton FR, Brand RE, Banks PA, Lewis MD, Disario JA, Gardner TB, Gelrud A, Amann ST, Baillie J, Money ME, O'Connell M, Whitcomb DC, Sherman S. Alcohol and smoking as risk factors in an epidemiology study of patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2011; **9**: 266-73; quiz e27 [PMID: 21029787 DOI: 10.1016/j.cgh.2010.10.015]

24 **Maisonneuve P**, Lowenfels AB, Müllhaupt B, Cavallini G, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L, Frulloni L, Ammann RW. Cigarette smoking accelerates progression of alcoholic chronic pancreatitis. *Gut* 2005; **54**: 510-514 [PMID: 15753536 DOI: 10.1136/gut.2004.039263]

25 **Tolstrup JS**, Kristiansen L, Becker U, Grønbaek M. Smoking and risk of acute and chronic pancreatitis among women and men: a population-based cohort study. *Arch Intern Med* 2009; **169**: 603-609 [PMID: 19307524 DOI: 10.1001/archinternmed.2008.601]

26 **Lévy P**, Mathurin P, Roqueplo A, Rueff B, Bernades P. A multidimensional case-control study of dietary, alcohol, and tobacco habits in alcoholic men with chronic pancreatitis. *Pancreas* 1995; **10**: 231-238 [PMID: 7624300]

27 **Gullo L**, Migliori M, Pezzilli R, Oláh A, Farkas G, Levy P, Arvanitakis C, Lankisch P, Beger H. An update on recurrent acute pancreatitis: data from five European countries. *Am J Gastroenterol* 2002; **97**: 1959-1962 [PMID: 12190160 DOI: 10.1111/j.1572-0241.2002.05907.x]

28 **Banks PA**, Freeman ML. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2379-2400 [PMID: 17032204 DOI: 10.1111/j.1572-0241.2006.00856.x]

29 **Bhasin DK**, Rana SS, Sidhu RS, Nagi B, Thapa BR, Poddar U, Gupta R, Sinha SK, Singh K. Clinical presentation and outcome of endoscopic therapy in patients with symptomatic chronic pancreatitis associated with pancreas divisum. *JOP* 2013; **14**: 50-56 [PMID: 23306335 DOI: 10.6092/1590-8577/1218]

30 **Patel MR**, Eppolito AL, Willingham FF. Hereditary pancreatitis for the endoscopist. *Therap Adv Gastroenterol* 2013; **6**: 169-179 [PMID: 23503650 DOI: 10.1177/1756283X12467565]

31 **Kamisawa T**, Shimosegawa T, Okazaki K, Nishino T, Watanabe H, Kanno A, Okumura F, Nishikawa T, Kobayashi K, Ichiya T, Takatori H, Yamakita K, Kubota K, Hamano H, Okamura K, Hirano K, Ito T, Ko SB, Omata M. Standard steroid treatment for autoimmune pancreatitis. *Gut* 2009; **58**: 1504-1507 [PMID: 19398440 DOI: 10.1136/gut.2008.172908]

32 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244]

33 **Chebli JM**, de Souza AF, Gaburri PD, Bastos KV, Ribeiro TC, Filho RJ, Chebli LA, Castro Ferreira LE. Prevalence and pathogenesis of duodenal ulcer in chronic alcoholic pancreatitis. *J Clin Gastroenterol* 2002; **35**: 71-74 [PMID: 12080230]

34 **Andrén-Sandberg A**, Ansorge C, Eiriksson K, Glomsaker T, Maleckas A. Treatment of pancreatic pseudocysts. *Scand J Surg* 2005; **94**: 165-175 [PMID: 16111100]

35 **Vijungco JD**, Prinz RA. Management of biliary and duodenal complications of chronic pancreatitis. *World J Surg* 2003; **27**: 1258-1270 [PMID: 14534824 DOI: 10.1007/s00268-003-7246-7]

36 **Kahl S**, Zimmermann S, Genz I, Schmidt U, Pross M, Schulz HU, Malfertheiner P. Biliary strictures are not the cause of pain in patients with chronic pancreatitis. *Pancreas* 2004; **28**: 387-390 [PMID: 15097855]

37 **Jadad AR**, Browman GP. The WHO analgesic ladder for cancer pain management. Stepping up the quality of its evaluation. *JAMA* 1995; **274**: 1870-1873 [PMID: 7500538]

38 **Anderson BJ**. What we don't know about paracetamol in children. *Paediatr Anaesth* 1998; **8**: 451-460 [PMID: 9836208]

39 **Thiagarajan P**, Jankowski JA. Aspirin and NSAIDs; benefits and harms for the gut. *Best Pract Res Clin Gastroenterol* 2012; **26**: 197-206 [PMID: 22542157 DOI: 10.1016/j.bpg.2012.01.007]

40 **Brock C**, Olesen SS, Olesen AE, Frøkjaer JB, Andresen T, Drewes AM. Opioid-induced bowel dysfunction: pathophysiology and management. *Drugs* 2012; **72**: 1847-1865 [PMID: 22950533 DOI: 10.2165/11634970-000000000-00000]

41 **Wilder-Smith CH**, Hill L, Osler W, O'Keefe S. Effect of tramadol and morphine on pain and gastrointestinal motor function in patients with chronic pancreatitis. *Dig Dis Sci* 1999; **44**: 1107-1116 [PMID: 10389680]

42 **Fioravanti B**, Vanderah TW. The ORL-1 receptor system: are there opportunities for antagonists in pain therapy? *Curr Top Med Chem* 2008; **8**: 1442-1451 [PMID: 18991730]

43 **De Schepper HU**, Cremonini F, Park MI, Camilleri M. Opioids and the gut: pharmacology and current clinical experience. *Neurogastroenterol Motil* 2004; **16**: 383-394 [PMID: 15305992 DOI: 10.1111/j.1365-2982.2004.00513.x]

44 **Sengupta JN**, Su X, Gebhart GF. Kappa, but not mu or delta, opioids attenuate responses to distention of afferent fibers innervating the rat colon. *Gastroenterology* 1996; **111**: 968-980 [PMID: 8831591]

45 **Staahl C**, Dimcevski G, Andersen SD, Thorsgaard N, Christrup LL, Arendt-Nielsen L, Drewes AM. Differential effect of opioids in patients with chronic pancreatitis: an experimental pain study. *Scand J Gastroenterol* 2007; **42**: 383-390 [PMID: 17354119 DOI: 10.1080/00365520601014414]

46 **Eisenach JC**, Carpenter R, Curry R. Analgesia from a peripherally active kappa-opioid receptor agonist in patients with chronic pancreatitis. *Pain* 2003; **101**: 89-95 [PMID: 12507703]

47 **Mercadante S**, Tirelli W, David F, Arcara C, Fulfaro F, Casuccio A, Gebbia V. Morphine versus oxycodone in pancreatic cancer pain: a randomized controlled study. *Clin J Pain* 2010;

26: 794-797 [PMID: 20973155]

48 **Drewes AM**, Jensen RD, Nielsen LM, Droney J, Christrup LL, Arendt-Nielsen L, Riley J, Dahan A. Differences between opioids: pharmacological, experimental, clinical and economical perspectives. *Br J Clin Pharmacol* 2013; **75**: 60-78 [PMID: 22554450 DOI: 10.1111/j.1365-2125.2012.04317.x]

49 **Niemann T**, Madsen LG, Larsen S, Thorsgaard N. Opioid treatment of painful chronic pancreatitis. *Int J Pancreatol* 2000; **27**: 235-240 [PMID: 10952406]

50 **Brush DE**. Complications of long-term opioid therapy for management of chronic pain: the paradox of opioid-induced hyperalgesia. *J Med Toxicol* 2012; **8**: 387-392 [PMID: 22983894 DOI: 10.1007/s13181-012-0260-0]

51 **Olesen SS**, Bouwense SA, Wilder-Smith OH, van Goor H, Drewes AM. Pregabalin reduces pain in patients with chronic pancreatitis in a randomized, controlled trial. *Gastroenterology* 2011; **141**: 536-543 [PMID: 21683078 DOI: 10.1053/j.gastro.2011.04.003]

52 **Finnerup NB**, Sindrup SH, Jensen TS. The evidence for pharmacological treatment of neuropathic pain. *Pain* 2010; **150**: 573-581 [PMID: 20705215 DOI: 10.1016/j.pain.2010.06.019]

53 **Tuchman M**, Barrett JA, Donevan S, Hedberg TG, Taylor CP. Central sensitization and Ca(V)$\alpha_2\delta$ ligands in chronic pain syndromes: pathologic processes and pharmacologic effect. *J Pain* 2010; **11**: 1241-1249 [PMID: 20472509 DOI: 10.1016/j.jpain.2010.02.024]

54 **Fink K**, Dooley DJ, Meder WP, Suman-Chauhan N, Duffy S, Clusmann H, Göthert M. Inhibition of neuronal Ca(2+) influx by gabapentin and pregabalin in the human neocortex. *Neuropharmacology* 2002; **42**: 229-236 [PMID: 11804619]

55 **Fehrenbacher JC**, Taylor CP, Vasko MR. Pregabalin and gabapentin reduce release of substance P and CGRP from rat spinal tissues only after inflammation or activation of protein kinase C. *Pain* 2003; **105**: 133-141 [PMID: 14499429]

56 **Bouwense SA**, Olesen SS, Drewes AM, Poley JW, van Goor H, Wilder-Smith OH. Effects of pregabalin on central sensitization in patients with chronic pancreatitis in a randomized, controlled trial. *PLoS One* 2012; **7**: e42096 [PMID: 22879908 DOI: 10.1371/journal.pone.0042096]

57 **Olesen SS**, Graversen C, Olesen AE, Frøkjaer JB, Wilder-Smith O, van Goor H, Valeriani M, Drewes AM. Randomised clinical trial: pregabalin attenuates experimental visceral pain through sub-cortical mechanisms in patients with painful chronic pancreatitis. *Aliment Pharmacol Ther* 2011; **34**: 878-887 [PMID: 21848870 DOI: 10.1111/j.1365-2036.2011.04802.x]

58 **Domino EF**. Taming the ketamine tiger. 1965. *Anesthesiology* 2010; **113**: 678-684 [PMID: 20693870 DOI: 10.1097/ALN.0b013e3181ed09a2]

59 **Woolf CJ**, Thompson SW. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain* 1991; **44**: 293-299 [PMID: 1828878]

60 **Noppers I**, Niesters M, Aarts L, Smith T, Sarton E, Dahan A. Ketamine for the treatment of chronic non-cancer pain. *Expert Opin Pharmacother* 2010; **11**: 2417-2429 [PMID: 20828267 DOI: 10.1517/14656566.2010.515978]

61 **Bouwense SA**, Buscher HC, van Goor H, Wilder-Smith OH. S-ketamine modulates hyperalgesia in patients with chronic pancreatitis pain. *Reg Anesth Pain Med* 2011; **36**: 303-307 [PMID: 21490522 DOI: 10.1097/AAP.0b013e3182177022]

62 **Okazaki K**, Yamamoto Y, Ito K. Endoscopic measurement of papillary sphincter zone and pancreatic main ductal pressure in patients with chronic pancreatitis. *Gastroenterology* 1986; **91**: 409-418 [PMID: 3721126]

63 **Xie JY**, Herman DS, Stiller CO, Gardell LR, Ossipov MH, Lai J, Porreca F, Vanderah TW. Cholecystokinin in the rostral ventromedial medulla mediates opioid-induced hyperalgesia and antinociceptive tolerance. *J Neurosci* 2005; **25**: 409-416 [PMID: 15647484 DOI: 10.1523/JNEUROSCI.4054-04.2005]

64 **Isaksson G**, Ihse I. Pain reduction by an oral pancreatic enzyme preparation in chronic pancreatitis. *Dig Dis Sci* 1983; **28**: 97-102 [PMID: 6825540]

65 **Slaff J**, Jacobson D, Tillman CR, Curington C, Toskes P. Protease-specific suppression of pancreatic exocrine secretion. *Gastroenterology* 1984; **87**: 44-52 [PMID: 6202586]

66 **Malesci A**, Gaia E, Fioretta A, Bocchia P, Ciravegna G, Cantor P, Vantini I. No effect of long-term treatment with pancreatic extract on recurrent abdominal pain in patients with chronic pancreatitis. *Scand J Gastroenterol* 1995; **30**: 392-398 [PMID: 7610357]

67 **Halgreen H**, Pedersen NT, Worning H. Symptomatic effect of pancreatic enzyme therapy in patients with chronic pancreatitis. *Scand J Gastroenterol* 1986; **21**: 104-108 [PMID: 3633631]

68 **Mössner J**, Secknus R, Meyer J, Niederau C, Adler G. Treatment of pain with pancreatic extracts in chronic pancreatitis: results of a prospective placebo-controlled multicenter trial. *Digestion* 1992; **53**: 54-66 [PMID: 1289173]

69 **Czakó L**, Takács T, Hegyi P, Prónai L, Tulassay Z, Lakner L, Döbrönte Z, Boda K, Lonovics J. Quality of life assessment after pancreatic enzyme replacement therapy in chronic pancreatitis. *Can J Gastroenterol* 2003; **17**: 597-603 [PMID: 14571298]

70 **Vecht J**, Symersky T, Lamers CB, Masclee AA. Efficacy of lower than standard doses of pancreatic enzyme supplementation therapy during acid inhibition in patients with pancreatic exocrine insufficiency. *J Clin Gastroenterol* 2006; **40**: 721-725 [PMID: 16940886]

71 **Brown A**, Hughes M, Tenner S, Banks PA. Does pancreatic enzyme supplementation reduce pain in patients with chronic pancreatitis: a meta-analysis. *Am J Gastroenterol* 1997; **92**: 2032-2035 [PMID: 9362186]

72 **Winstead NS**, Wilcox CM. Clinical trials of pancreatic enzyme replacement for painful chronic pancreatitis--a review. *Pancreatology* 2009; **9**: 344-350 [PMID: 19451744 DOI: 10.1159/000212086]

73 **Foster E**, Leung J. Pharmacotherapy for the prevention of post-ERCP pancreatitis. *Am J Gastroenterol* 2007; **102**: 52-55 [PMID: 17266688 DOI: 10.1111/j.1572-0241.2006.00950.x]

74 **Malfertheiner P**, Mayer D, Büchler M, Domínguez-Muñoz JE, Schiefer B, Ditschuneit H. Treatment of pain in chronic pancreatitis by inhibition of pancreatic secretion with octreotide. *Gut* 1995; **36**: 450-454 [PMID: 7698708]

75 **Lieb JG**, Shuster JJ, Theriaque D, Curington C, Cintrón M, Toskes PP. A pilot study of Octreotide LAR vs. octreotide tid for pain and quality of life in chronic pancreatitis. *JOP* 2009; **10**: 518-522 [PMID: 19734628]

76 **Burton F**, Alkaade S, Collins D, Muddana V, Slivka A, Brand RE, Gelrud A, Banks PA, Sherman S, Anderson MA, Romagnuolo J, Lawrence C, Baillie J, Gardner TB, Lewis MD, Amann ST, Lieb JG, O'Connell M, Kennard ED, Yadav D, Whitcomb DC, Forsmark CE. Use and perceived effectiveness of non-analgesic medical therapies for chronic pancreatitis in the United States. *Aliment Pharmacol Ther* 2011; **33**: 149-159 [PMID: 21083584 DOI: 10.1111/j.1365-2036.2010.04491.x]

77 **Uden S**, Bilton D, Guyan PM, Kay PM, Braganza JM. Rationale for antioxidant therapy in pancreatitis and cystic fibrosis. *Adv Exp Med Biol* 1990; **264**: 555-572 [PMID: 2244539]

78 **Bhardwaj P**, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. *Gastroenterology* 2009; **136**: 149-159.e2 [PMID: 18952082 DOI: 10.1053/j.gastro.2008.09.028]

79 **Siriwardena AK**, Mason JM, Sheen AJ, Makin AJ, Shah NS. Antioxidant therapy does not reduce pain in patients with chronic pancreatitis: the ANTICIPATE study. *Gastroenterology* 2012; **143**: 655-63.e1 [PMID: 22683257 DOI: 10.1053/j.gastro.2012.05.046]

80 **Forsmark CE**, Liddle RA. The challenging task of treating painful chronic pancreatitis. *Gastroenterology* 2012; **143**:

533-535 [PMID: 22841737 DOI: 10.1053/j.gastro.2012.07.029]

81 **Cartmell MT**, O'Reilly DA, Porter C, Kingsnorth AN. A double-blind placebo-controlled trial of a leukotriene receptor antagonist in chronic pancreatitis in humans. *J Hepatobiliary Pancreat Surg* 2004; **11**: 255-259 [PMID: 15368110 DOI: 10.1007/s00534-004-0890-y]

82 **Levenick JM**, Andrews CL, Purich ED, Gordon SR, Gardner TB. A phase II trial of human secretin infusion for refractory type B pain in chronic pancreatitis. *Pancreas* 2013; **42**: 596-600 [PMID: 23548879 DOI: 10.1097/MPA.0b013e318273f3ec]

83 **Olesen SS**, Graversen C, Bouwense SA, van Goor H, Wilder-Smith OH, Drewes AM. Quantitative sensory testing predicts pregabalin efficacy in painful chronic pancreatitis. *PLoS One* 2013; **8**: e57963 [PMID: 23469256 DOI: 10.1371/journal.pone.0057963]

84 **Lane NE**, Schnitzer TJ, Birbara CA, Mokhtarani M, Shelton DL, Smith MD, Brown MT. Tanezumab for the treatment of pain from osteoarthritis of the knee. *N Engl J Med* 2010; **363**: 1521-1531 [PMID: 20942668 DOI: 10.1056/NEJMoa0901510]

85 **Pal M**, Angaru S, Kodimuthali A, Dhingra N. Vanilloid receptor antagonists: emerging class of novel anti-inflammatory agents for pain management. *Curr Pharm Des* 2009; **15**: 1008-1026 [PMID: 19275664]

86 **Friess H**, Zhu ZW, di Mola FF, Kulli C, Graber HU, Andren-Sandberg A, Zimmermann A, Korc M, Reinshagen M, Büchler MW. Nerve growth factor and its high-affinity receptor in chronic pancreatitis. *Ann Surg* 1999; **230**: 615-624 [PMID: 10561084]

P- Reviewers: Abraham P, Barauskas G, Xu CF
S- Editor: Zhai HH **L- Editor**: A **E- Editor**: Zhang DN

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7302

World J Gastroenterol 2013 November 14; 19(42): 7302-7307
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

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Pharmacological challenges in chronic pancreatitis

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Supported by The Danish Council for Strategic Research

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Received: June 19, 2013 Revised: October 2, 2013
Accepted: October 19, 2013
Published online: November 14, 2013

Abstract

Drug absorption in patients with chronic pancreatitis might be affected by the pathophysiology of the disease. The exocrine pancreatic insufficiency is associated with changes in gastrointestinal intraluminal pH, motility disorder, bacterial overgrowth and changed pancreatic gland secretion. Together these factors can result in malabsorption and may also affect the efficacy of pharmacological intervention. The lifestyle of chronic pancreatitis patients may also contribute to gastrointestinal changes. Many patients limit their food intake because of the pain caused by eating and in some cases food intake is more or less substituted with alcohol, tobacco and coffee. Alcohol and drug interaction are known to influence the pharmacokinetics by altering either drug absorption or by affecting liver metabolism. Since patients suffering from chronic pancreatitis experience severe pain, opioids are often prescribed as pain treatment. Opioids have intrinsic effects on gastrointestinal motility and hence can modify the absorption of other drugs taken at the same time. Furthermore, the increased fluid absorption caused by opioids will decrease water available for drug dissolution and may hereby affect absorption of the drug. As stated above many factors can influence drug absorption and metabolism in patients with chronic pancreatitis. The factors may not have clinical relevance, but may explain inter-individual variations in responses to a given drug, in patients with chronic pancreatitis.

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Key words: Pharmacology; Absorption; Metabolism; Chronic pancreatitis; Treatment

Core tip: In patients with chronic pancreatitis several pathophysiological factors can account for malabsorption and may also affect the efficacy of pharmacological intervention by reduced drug absorption. For example it can be speculated that changes in gastrointestinal intraluminal pH, motility disorder, bacterial overgrowth and changed pancreatic gland secretion may contribute. The lifestyle of chronic pancreatitis patients may also be a factor to gastrointestinal changes. The factors may not have clinical relevance, but may explain inter-individual variations in responses to a given drug, in patients with chronic pancreatitis.

Olesen AE, Brokjaer A, Fischer IW, Larsen IM. Pharmacological challenges in chronic pancreatitis. *World J Gastroenterol* 2013; 19(42): 7302-7307 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7302.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7302

INTRODUCTION

Chronic pancreatitis is a persistent inflammation of the pancreas that results in irreversible morphological changes and impairment of both exocrine and endocrine functions[1]. The etiology of chronic pancreatitis is multifactorial and the risk factors include alcohol and nicotine consumption, hereditary factors, efferent duct obstructions, immunological factors or rare metabolic disorders[2]. It is well known that pancreatitis patients suffer from malabsorption[3-5] and it could be hypothesized that this would also affect drug absorption.

Drug absorption in patients with gastrointestinal disorders can be influenced by alterations in several factors. For example; gastric and intestinal motility, changes in the mucosal surface area available for drug absorption, and altered physical and chemical properties of the intestinal luminal content. These properties are usually changed in combination and the degree of each factor impact is dependent on the duration and severity of the disease[6]. Despite this, most of the data about bioavailability of orally administered drugs are obtained from healthy individuals.

The knowledge about drug absorption in patients with chronic pancreatitis is limited. It has been demonstrated that the pharmacokinetic profile of pregabalin was not extensively affected by chronic pancreatitis[7]. However, inter-individual variations were found and several factors affecting drug pharmacokinetic profiles in patients with chronic pancreatitis may be relevant to consider.

This aim of this review was therefore to evaluate different factors which could possibly affect drug absorption in chronic pancreatitis patients leading to pharmacological challenges in this patient group. Moreover, suggestions on how to diminish the impact of these factors will be provided.

GASTROINTESTINAL PHYSIOLOGICAL CHANGES THAT MAY AFFECT DRUG EFFECTS

Two primary factors influencing bioavailability of orally administered medications is the amount of drug absorbed, and metabolism by the liver. Hence, any factor influencing the gastrointestinal tract can alter drug absorption, such as gastric pH, regional blood flow, mucosal surface area and gut motility[8].

Drug absorption

Chronic pancreatitis is a clinical condition in which exocrine pancreatic insufficiency occur leading to secondary maldigestion. Several causes of exocrine pancreatic insufficiency may be associated with changes in gastrointestinal physiology such as: (1) changes in gastrointestinal intraluminal pH; (2) motility disorders; (3) bacterial overgrowth; and (4) pancreatic secretion.

Together these factors can result in malabsorption and may also affect the efficacy of pharmacological treatment in exocrine pancreatic insufficiency[8]. The main clinical manifestation of exocrine pancreatic insufficiency is fat malabsorption, identified as steatorrhea[4]. Steatorrhea leads to deficit of fat-soluble vitamins (A, D, E and K) with consequent clinical manifestations[4]. Moreover, fat malabsorption may affect absorption of lipophilic drug formulations and as such deserves particular attention[6].

Changes in intraluminal pH: Low intraluminal pH in the upper small intestine might be a factor in the pathogenesis of fecal loss of bile acids in pancreatic insufficiency[4]. The drug's ability to cross membranes is determined by the environmental pH and the acid dissociation constant (pKa). Therefore, decreased intraluminal pH may affect drug absorption. However, this may be of variable importance, as changes in intraluminal pH might be negligible in some cases and more pronounced in others.

Motility disorders: The rate of gastric emptying and intestinal transit is abnormal in patients with chronic pancreatitis and this may affect the efficacy of treatments[8]. Gastric emptying can be accelerated due to diarrhea[4] or decreased by, *e.g.*, opioids. Because most drugs are absorbed through the small intestines, delayed gastric emptying will prolong the time to peak concentration and delay the onset of the action of a drug[9]. Clinicians should be aware that delayed gastric emptying can delay the onset of action of a medication if administered orally[9]. On the other hand accelerated gastric emptying will have the opposite effect. Taken together gastric emptying is likely to be a rate limiting step in drug absorption unless normal absorption (prolonged release) is slow[10].

Bacterial overgrowth: One mechanism which has been hypothesized between maldigestion and intestinal alterations relates to bacterial overgrowth in the small intestine. It has been speculated that lack of coordination between motor activity and peak of secretory activity in the gastrointestinal tract may reduce the effectiveness of the "housekeeper" function and thereby contribute to the intestinal bacterial overgrowth often observed in patients with chronic pancreatitis[11]. Bacterial overgrowth might either contribute to diarrhea or account for the persistence of diarrhea[4]. Moreover, bacterial overgrowth might give rise to bile acid malabsorption and changes in intestinal permeability[4,12]. Bacterial overgrowth may interfere with the normal intestinal environment and lead to atrophic mucosa with structural abnormalities which can decrease drug absorption[13].

Pancreatic gland secretion: The pancreatic gland normally secretes more than 2 L of juice per day (high protein content) with enzymes able to digest lipids, proteins and carbohydrates. Patients with exocrine pancreatic insufficiency exhibit decreased pancreatic bicarbonate secretion resulting in reduction in duodenal pH postprandial, leading to inactivation of orally administered exogenous enzymes[6]. In the same line acidic inactivation of pancreatic enzymes is considered a significant reason for

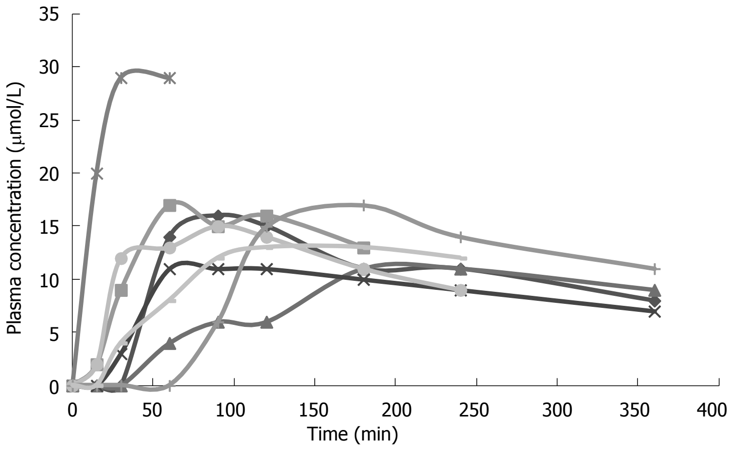

Figure 1 Absorption of pregabalin in eight female patients with chronic pancreatitis. Curves illustrate the variance in absorption of pregabalin after 75 mg pregabalin (oral capsule). Especially one patient varied from other subjects by having increased plasma concentration of pregabalin. Not a single cause could explain this outlier, but it could be a combination of several factors suggested.

failure of drug therapy[8].

A combination of the above mentioned factors may affect drug absorption in patients with chronic pancreatitis. Figure 1 illustrates the variance in absorption of pregabalin in a group of women with chronic pancreatitis.

Drug elimination

To our knowledge only one study investigated hepatic drug metabolism in patients with chronic pancreatitis and found reduced drug elimination capacity in this group of patients[14]. The results were most probably explained by the patients' general state, with a fairly overt malnutrition, although theoretically, a subclinical, probably alcohol-induced, liver affection could not be ruled out[14]. Interestingly, the study results yielded the need for caution upon administration of drugs that are biotransformed in the liver.

Thus, to optimize the efficacy of pharmaceutical treatment, the management of exocrine pancreatic insufficiency should ideally be individually tailored to account for both the underlying cause and any associated disturbance in gastrointestinal physiology.

Lifestyle

The gastrointestinal physiological changes in chronic pancreatitis can be further affected by patients' lifestyle. Many patients limit their food intake because of the pain caused by eating and in some cases food intake is more or less substituted with tobacco and coffee[15]. Due to malabsorption and lifestyle factors, chronic pancreatitis patients are likely to have a lower body mass index than those not suffering from this disease[15]. Thus, body mass index is an easy accessible but important factor to consider in dose decision in these patients, as drug disposition might be affected. This is especially important when drugs with a narrow therapeutic index, such as, *e.g.*, warfarin and digoxin are prescribed[14].

Furthermore, decreased food intake, could be an important factor in several cases of chronic pancreatitis as food intake will affect some of the physiological processes, *e.g.*, changes in pH, reduced gastric emptying time, increased gall secretion, increased motility, increased gastrointestinal and liver blood flow. Most of these processes are already affected by the pancreatitis and hence these changes could possibly affect drug bioavailability. Consequently, reduced food intake in itself will worsen gastrointestinal physiological processes.

Insufficient food intake due to nausea, anorexia or alcoholism may also be of some significance[16]. Alcohol abuse is a well known etiological factor (alcoholic chronic pancreatitis) and the lifestyle of the alcoholic chronic pancreatitis patient group is in general characterized by excessive alcohol consumption and smoking. Together, these factors can be further accompanied by insufficient food intake and will eventually also lead to malnutrition.

Alcohol can be used as a central nervous system depressant to relieve pain and concerns about the disease. Therefore many patients with chronic pancreatitis continue their alcohol consumption throughout disease progression. In relation to alcohol induced chronic pancreatitis it is relevant to consider how the lifestyle with regard to alcohol- and alcohol related dietary habits may provide pharmacologically challenges. The most frequent pharmacological interaction is the combination of alcohol with other depressors of the central nervous system[17]. Moreover, alcohol drug interactions can influence the pharmacokinetics of a drug by altering the drug absorption or by affecting the liver metabolism. The effect will vary with, *e.g.*, the amount of alcohol consumed, the nature of the drug, the dosage and how the drug is administered. There are various interactions between alcohol and drugs. Antihistamines, analgesics and antidepressants are examples of drugs which may interact with alcohol.

The liver is the primary site of drug metabolism and the cytochrome P450 mixed-function oxidase enzyme system, is primarily responsible for this process. Alcohol inhibits the oxidation of drugs by cytochrome P450 isoenzyme (CYP2E1). In contrast, chronic alcohol consumption will cause induction of the CYP2E1 leading to increased drug metabolism. Previously, concerns have been raised regarding interactions of paracetamol and alcohol. However, paracetamol interaction is only a problem when ingestion of alcohol is suddenly stopped. Consequently, the hepatic glutathione is unable to detoxify which leads to irreversible hepatic damage. Therefore, in chronic alco-

Table 1 Pathophysiological and lifestyle related effects on drug absorption

Pathophysiology	Effect on drug absorption
Low intraluminal pH	The drugs ability to cross the luminal wall
Motility disorder	Delayed drug effect
Bacterial overgrowth	Decreased absorption due to diarrhea or structural abnormalities
Pancreatic secretion	Lack of enzymes leads to inactivation of pro-drugs
Steatorrhea	Problems with absorption of lipophilic drugs
Low body mass index	Increased plasma concentration

holic patients the consumption of alcohol should not be suspended on prescribing paracetamol[17]. Additionally, extensive alcohol consumption may result in gastric mucosal injury and hereby further affect drug absorption[8].

Smoking may increase hepatic drug metabolism to a significant extent[14] and may in addition result in induction of the cytochrome oxidases[18]. It has also been demonstrated that body mass index is associated with cigarette smoking in chronic alcoholic-associated pancreatitis patients; the more cigarettes smoked per day, the lower the mean body mass index[19]. This is in line with the hypothesis that alcohol consumption and smoking can cause insufficient food intake and hereby affecting drug absorptions.

Abdominal pain is usually the symptom that causes patients with chronic pancreatitis to seek medical attention. In parallel, they can have a history of alcoholic abuse making opioids, with their associated abuse potential together with other side effects as, for example, bowel dysfunction, less suitable for these patients and other treatment regimens should be considered[7]. Recently it was demonstrated that pregabalin was superior to placebo for attenuation of experimental visceral pain in chronic pancreatitis patients[20]. Therefore, pregabalin may be used to treat pain in patients with chronic pancreatitis when there is a conflict or concern with abuse.

A summary of pathophysiological effects on drug absorption is given in Table 1.

MANAGEMENT IN CHRONIC PANCREATITIS

The physiological processes affected by chronic pancreatitis are widespread. This section will focus on how to treat the pathophysiology of chronic pancreatitis in order to reduce the impact of factors possibly affecting drug absorption.

Pharmacological management

Enzyme therapy: The main goal with enzyme treatment is to achieve optimal enzyme activity in the duodenum[21] and hereby improve the nutritional status, preventing weight loss, vitamin deficiencies and exocrine pancreatic insufficiency related symptoms, resulting in steatorrhea. The most widely used enzyme preparation is porcine pancreatin. The preparation contains a mixture of protease, lipase and amylase[22]. Löhr *et al*[23] analyzed the effectiveness of different preparations and concluded that overall pancreatin preparation replacements must contain high lipase activity. Lipase of porcine pancreatin is destroyed by protease and acids, thus it is necessary to protect the pancreatin against the influence of gastric acids. Another factor that is of great importance is the particle size and the rate of which the porcine pancreatin is released into the duodenum. The best particle size is assumed to be a diameter of ≤ 2 mm, since these particles leaves the stomach at the same time as solid food. The enzymes should be released within 30 min[24]. Pancreatic enzyme supplements improve fat absorption[25], and hence reduces steatorrhea[26] and this may have beneficial effects on drug absorption. In contrast high-dose enzyme replacement therapy with or without gastric acid suppression may cause additional challenges related to drug absorption and interactions if additional drug therapy is required[6]. Thus, enzyme treatment can either enhance or complicate drug absorption in different aspects and this should be considered in the pharmacological management of clinical symptoms.

Endoscopic therapy or surgical management

More invasive treatment is recommended for patients with pancreatic duct stones and pancreatic obstruction in whom standard medical therapy is not sufficient. The goals of endoscopy and surgery are to decompress ducts, dilate stricture with stent placement and preserve pancreatic tissue and adjacent organs[27,28]. Endoscopic therapy should be the first-line option because it is less invasive than surgery[29]. Surgery should be the first-line option in patients in whom endoscopic therapy failed or those with pancreatic mass with suspicion of malignancy[29]. It has been assumed that invasive procedures designed to improve drainage of the main pancreatic duct, will result in decreased pain. It could therefore be hypothesized that pain attenuation can improve the lifestyle and hereby indirectly affecting drug absorption. There is consensus that endoscopical and surgical management have both benefits and harms[26] and it has been suggested that management may include medical, endoscopic and surgical approaches with the interaction between various specialties, calling for a concerted multidisciplinary approach[28]. Moreover, as surgery has a low rate of success with attendant morbidity, mortality and slow recovery rates, it is not considered an option for optimizing absorption of drug in the pharmacological management.

Lifestyle changes

Patients with chronic pancreatitis are often advised to eat small meals with low-fat content (< 20 g of fat) in an attempt to decrease the need of pancreatic secretion[30]. Low-fat diets decreases the amount of overall fat presented to the intestine for digestion and absorption, and may be helpful alleviating steatorrhea[26] and hereby could lead to better conditions for some drug absorptions.

However, a systematic review of benefits and harms of low-fat diet in chronic pancreatitis found no studies of sufficient quality to confirm the effect of low-fat diet[31]. Thus, if people are given pancreatic enzyme supplements, they are usually advised to maintain a normal diet, as there is no need to lower fat intake alongside enzyme supplementation[31].

Overeating is dissuaded, instead smaller meals on a more frequent basis is preferred. Frequent meals will also benefit gastric motility and result in more normal gastrointestinal conditions, and hereby improve drug absorption. The restriction in fat intake should be monitored by a dietitian who follows the total caloric intake and the diet should compensate the loss in caloric intake by carbohydrate-enriched diet. On a carbohydrate-enriched diet 65%-70% of the total daily energy intake should derive from carbohydrate. Hereby, it is possible for the patient to gain weight, which again will be beneficial in several ways, *e.g.*, in relation to drug absorption, where body weight and body composition is directly related to drug distribution volumes.

In general alcohol consumption and smoking should be diminished or avoided to reduce the impact on the pharmacokinetic profiles. Total alcohol abstinence is only recommended for patients whose chronic pancreatitis is derived from alcohol abuse.

ADDITIONAL GASTROINTESTINAL CHANGES CAUSED BY OPIOIDS

Patients with painful chronic pancreatitis are often treated with opioids which lead to diverse issues and alterations in the gastrointestinal tract. Opioids have intrinsic effects on gastrointestinal motility and can modify the absorption of other drugs taken at the same time[10]. It has been demonstrated that, *e.g.*, tramadol is an effective oral opioid analgesic for reducing pain in people with chronic pancreatitis, but it is also associated with gastrointestinal adverse effect[26]. Opioids will affect opioid receptors in the enteric nervous system and will cause changes in motility, sphincter function and secretion which affect absorption leading to opioid induced bowel dysfunction[32].

Opioid induced decreased motility occurs throughout the entire gastrointestinal tract. In the circular muscle in the small and large intestine, opioids induce increased resting contractile tone and decreases tonic inhibition of the muscle tone, which leads to increased tone in the circular muscle layer. This is accompanied by occasional occurrence of high-amplitude, non-propulsive phasic contractions enhanced by rhythmic contractions and associated changes in smooth muscle electrical activity. These motility abnormalities result in decreased propulsive forward peristalsis, and increased segmental contraction which in clinical settings manifests as constipation, abdominal cramps and gut spasm[33-35]. An additional consequence of this peristaltic disruption is stasis of luminal contents, which leads to increased passive fluid absorption[36]. Furthermore, intestinal fluid secretion is inhibited by opioids directly *via* the enteric nervous system. In the sympathetic nervous system opioids also increase activity and thereby decrease the secretion. Serotonin and noradrenaline as terminal transmitters seems to dominate the local effect[37,38]. An overall decreased gut secretion of intestinal fluids takes place and together leads to harder and dryer stools[36-38]. Therefore, patients treated with opioids might have even more pronounced decreased motility which will complicate drug absorption further. Furthermore, as solid drug forms must dissolve before absorption can occur; dissolution rate determines availability of the drug for absorption. The increased fluid absorption caused by opioid effects will decrease water available for drug dissolution and may hereby affect absorption. If dissolution is slower than absorption it becomes the rate-limiting step.

CONCLUSION

Several factors might affect drug absorption and metabolism in patients with chronic pancreatitis: gastric pH, regional blood flow, mucosal surface area and gut motility. The impact of these factors on drug absorption may be reduced by treating the pathophysiology of chronic pancreatitis. Treatment can be pharmacological management, enzyme therapy, endoscopic therapy or surgical management. Moreover, as gastrointestinal physiological changes in chronic pancreatitis can be affected by patients' lifestyle, lifestyle changes may lead to more optimal drug absorption. However, issues raised in this review may not have clinical relevance, but could explain part of the variation observed in drug effects in this patient group.

REFERENCES

1 **Chen JM**, Férec C. Genetics and pathogenesis of chronic pancreatitis: the 2012 update. *Clin Res Hepatol Gastroenterol* 2012; **36**: 334-340 [PMID: 22749696 DOI: 10.1016/j.clinre.2012.05.003]

2 **Yadav D**, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1252-1261 [PMID: 23622135 DOI: 10.1053/j.gastro.2013.01.068]

3 **Hammer HF**. Pancreatic exocrine insufficiency: diagnostic evaluation and replacement therapy with pancreatic enzymes. *Dig Dis* 2010; **28**: 339-343 [PMID: 20814209 DOI: 10.1159/000319411]

4 **Pezzilli R**. Chronic pancreatitis: maldigestion, intestinal ecology and intestinal inflammation. *World J Gastroenterol* 2009; **15**: 1673-1676 [PMID: 19360910]

5 **Forsmark CE**. Chronic pancreatitis and malabsorption. *Am J Gastroenterol* 2004; **99**: 1355-1357 [PMID: 15233678 DOI: 10.1111/j.1572-0241.2004.70661.x]

6 **Dressman JB**, Lennernäs H. Oral Drug Absorption, Prediction and Assessment. New York: Marcel Dekker Inc., 2005

7 **Olesen AE**, Olofsen E, Olesen SS, Staahl C, Andresen T, Dahan A, Drewes AM. The absorption profile of pregabalin in chronic pancreatitis. *Basic Clin Pharmacol Toxicol* 2012; **111**: 385-390 [PMID: 22716224 DOI: 10.1111/j.1742-7843.2012.00914.x]

8 **Bruno MJ**, Haverkort EB, Tytgat GN, van Leeuwen DJ. Maldigestion associated with exocrine pancreatic insufficiency: implications of gastrointestinal physiology and properties of enzyme preparations for a cause-related and patient-tailored treatment. *Am J Gastroenterol* 1995; **90**: 1383-1393 [PMID:

7661155]
9 **Smith BS**, Yogaratnam D, Levasseur-Franklin KE, Forni A, Fong J. Introduction to drug pharmacokinetics in the critically ill patient. *Chest* 2012; **141**: 1327-1336 [PMID: 22553267 DOI: 10.1378/chest.11-1396]
10 **Prescott LF**. Gastric emptying and drug absorption. *Br J Clin Pharmacol* 1974; **1**: 189-190 [PMID: 22454945]
11 **Pieramico O**, Dominguez-Muñoz JE, Nelson DK, Böck W, Büchler M, Malfertheiner P. Interdigestive cycling in chronic pancreatitis: altered coordination among pancreatic secretion, motility, and hormones. *Gastroenterology* 1995; **109**: 224-230 [PMID: 7540998]
12 **Mathias JR**, Clench MH. Review: pathophysiology of diarrhea caused by bacterial overgrowth of the small intestine. *Am J Med Sci* 1985; **289**: 243-248 [PMID: 3890541]
13 **Domínguez-Muñoz JE**. Pancreatic exocrine insufficiency: diagnosis and treatment. *J Gastroenterol Hepatol* 2011; **26** Suppl 2: 12-16 [PMID: 21323992 DOI: 10.1111/j.1440-1746.2010.06600.x]
14 **Andersen V**, Sonne J, Larsen S. Antipyrine, oxazepam, and indocyanine green clearance in patients with chronic pancreatitis and healthy subjects. *Scand J Gastroenterol* 1999; **34**: 813-817 [PMID: 10499483]
15 **Turner RC**, Brazionis LB, McDermott R. Intake patterns of food nutrients and other substances associated with chronic pancreatitis. *Pancreatology* 2013; **13**: 33-37 [PMID: 23395567 DOI: 10.1016/j.pan.2012.12.004]
16 **Armbrecht U**. [Chronic pancreatitis: weight loss and poor physical performance - experience from a specialized rehabilitation centre]. *Rehabilitation (Stuttg)* 2001; **40**: 332-336 [PMID: 11742423 DOI: 10.1055/s-2001-18966]
17 **Gómez-Moreno G**, Guardia J, Cutando A. Interaction of paracetamol in chronic alcoholic patients. Importance for odontologists. *Med Oral Patol Oral Cir Bucal* 2008; **13**: E235-E238 [PMID: 18379447]
18 **Acheson DW**, Hunt LP, Rose P, Houston JB, Braganza JM. Factors contributing to the accelerated clearance of theophylline and antipyrine in adults with exocrine pancreatic disease. *Clin Sci (Lond)* 1989; **76**: 377-385 [PMID: 2714050]
19 **Talamini G**, Vaona B, Bassi C, Bovo P, Damoc T, Mastromauro M, Falconi M, Vantini I, Cavallini G, Pederzoli P. Alcohol intake, cigarette smoking, and body mass index in patients with alcohol-associated pancreatitis. *J Clin Gastroenterol* 2000; **31**: 314-317 [PMID: 11129273]
20 **Olesen SS**, Graversen C, Olesen AE, Frøkjaer JB, Wilder-Smith O, van Goor H, Valeriani M, Drewes AM. Randomised clinical trial: pregabalin attenuates experimental visceral pain through sub-cortical mechanisms in patients with painful chronic pancreatitis. *Aliment Pharmacol Ther* 2011; **34**: 878-887 [PMID: 21848870 DOI: 10.1111/j.1365-2036.2011.04802.x]
21 **Ferrone M**, Raimondo M, Scolapio JS. Pancreatic enzyme pharmacotherapy. *Pharmacotherapy* 2007; **27**: 910-920 [PMID: 17542772 DOI: 10.1592/phco.27.6.910]
22 **Sikkens EC**, Cahen DL, Kuipers EJ, Bruno MJ. Pancreatic enzyme replacement therapy in chronic pancreatitis. *Best Pract Res Clin Gastroenterol* 2010; **24**: 337-347 [PMID: 20510833 DOI: 10.1016/j.bpg.2010.03.006]
23 **Löhr JM**, Hummel FM, Pirilis KT, Steinkamp G, Körner A, Henniges F. Properties of different pancreatin preparations used in pancreatic exocrine insufficiency. *Eur J Gastroenterol Hepatol* 2009; **21**: 1024-1031 [PMID: 19352190 DOI: 10.1097/MEG.0b013e328328f414]
24 **Mössner J**, Keim V. Pancreatic enzyme therapy. *Dtsch Arztebl Int* 2010; **108**: 578-582 [PMID: 21904592 DOI: 10.3238/arztebl.2011.0578]
25 **Waljee AK**, Dimagno MJ, Wu BU, Schoenfeld PS, Conwell DL. Systematic review: pancreatic enzyme treatment of malabsorption associated with chronic pancreatitis. *Aliment Pharmacol Ther* 2009; **29**: 235-246 [PMID: 19035969 DOI: 10.1111/j.1365-2036.2008.03885.x]
26 **Kocher HM**, Kadaba R. Chronic pancreatitis. *Clin Evid (Online)* 2011; **2011**: [PMID: 22189345]
27 **Banks PA**, Conwell DL, Toskes PP. The management of acute and chronic pancreatitis. *Gastroenterol Hepatol (N Y)* 2010; **6**: 1-16 [PMID: 20567557]
28 **Oza VM**, Kahaleh M. Endoscopic management of chronic pancreatitis. *World J Gastrointest Endosc* 2013; **5**: 19-28 [PMID: 23330050 DOI: 10.4253/wjge.v5.i1.19]
29 **Jabłońska B**. Is endoscopic therapy the treatment of choice in all patients with chronic pancreatitis? *World J Gastroenterol* 2013; **19**: 12-16 [PMID: 23326157 DOI: 10.3748/wjg.v19.i1.12]
30 **Shea JC**, Hopper IK, Blanco PG, Freedman SD. Advances in nutritional management of chronic pancreatitis. *Curr Gastroenterol Rep* 2000; **2**: 323-326 [PMID: 10981031]
31 **Kocher HM**, Froeling FE. Chronic pancreatitis. *Clin Evid (Online)* 2008; **2008**: [PMID: 19445788]
32 **Brock C**, Olesen SS, Olesen AE, Frøkjaer JB, Andresen T, Drewes AM. Opioid-induced bowel dysfunction: pathophysiology and management. *Drugs* 2012; **72**: 1847-1865 [PMID: 22950533 DOI: 10.2165/11634970-000000000-00000]
33 **Telford GL**, Condon RE, Szurszewski JH. Opioid receptors and the initiation of migrating myoelectric complexes in dogs. *Am J Physiol* 1989; **256**: G72-G77 [PMID: 2563201]
34 **Frantzides CT**, Cowles V, Salaymeh B, Tekin E, Condon RE. Morphine effects on human colonic myoelectric activity in the postoperative period. *Am J Surg* 1992; **163**: 144-148; discussion 144-148 [PMID: 1733363]
35 **Sarna SK**, Otterson MF. Small intestinal amyogenesia and dysmyogenesia induced by morphine and loperamide. *Am J Physiol* 1990; **258**: G282-G289 [PMID: 1968317]
36 **Thomas J**. Opioid-induced bowel dysfunction. *J Pain Symptom Manage* 2008; **35**: 103-113 [PMID: 17981003]
37 **Wood JD**, Galligan JJ. Function of opioids in the enteric nervous system. *Neurogastroenterol Motil* 2004; **16** Suppl 2: 17-28 [PMID: 15357848]
38 **De Luca A**, Coupar IM. Insights into opioid action in the intestinal tract. *Pharmacol Ther* 1996; **69**: 103-115 [PMID: 8984506]

P- Reviewers: Hardt PD, Hackert T, Lee SW **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Zhang DN

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World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7308

World J Gastroenterol 2013 November 14; 19(42): 7308-7315
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

Asbjørn Mohr Drewes, MD, PhD, DMSc, Professor, *Series Editor*

Endoscopic management of complications of chronic pancreatitis

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Received: June 10, 2013 **Revised:** July 15, 2013
Accepted: July 17, 2013
Published online: November 14, 2013

Abstract

Pseudocysts and biliary obstructions will affect approximately one third of patients with chronic pancreatitis (CP). For CP-related, uncomplicated, pancreatic pseudocysts (PPC), endoscopy is the first-choice therapeutic option. Recent advances have focused on endosonography-guided PPC transmural drainage, which tends to replace the conventional, duodenoscope-based coma immediately approach. Ancillary material is being tested to facilitate the endosonography-guided procedure. In this review, the most adequate techniques depending on PPC characteristics are presented along with supporting evidence. For CP-related biliary obstructions, endoscopy and surgery are valid therapeutic options. Patient co-morbidities (*e.g.*, portal cavernoma) and expected patient compliance to repeat endoscopic procedures are important factors when selecting the most adapted option. Malignancy should be reasonably ruled out before embarking on the endoscopic treatment of presumed CP-related biliary strictures. In endoscopy, the gold standard technique consists of placing simultaneous, multiple, side-by-side, plastic stents for a one-year period. Fully covered self-expandable metal stents are challenging this method and have provided 50% mid-term success.

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Key words: Biliary stricture; Chronic pancreatitis; Pseudocyst; Endoscopic retrograde cholangio-pancreatography; Endoscopic ultrasonography; Stent

Core tip: Endoscopy is the first-choice treatment of pancreatic pseudocysts. The transduodenal route may be preferable over the transgastric route. Two transmural double pigtail stents should be left for at least 2 mo. In the case of a disconnected pancreatic tail, secretin-enhanced magnetic resonance pancreatography should be obtained to decide about stent removal. Biliary strictures should be thoroughly investigated to rule out malignancy. To this aim, improved methods of biliary sampling have become available. Even with multiple biliary stents, potentially fatal cholangitis is frequent in the absence of regular stent revision. Fully covered self-expandable metal stents have provided 50% mid-term success.

Dumonceau JM, Macias-Gomez C. Endoscopic management of complications of chronic pancreatitis. *World J Gastroenterol* 2013; 19(42): 7308-7315 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7308.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7308

INTRODUCTION

Common local complications of chronic pancreatitis (CP) include pancreatic pseudocysts (PPCs) and biliary obstructions. These two complications develop in patients during the course of CP at a rate of 20%-40% for PPCs and 3%-23% for biliary obstructions[1,2]. PPCs consist of a collection of pancreatic juice enclosed by a wall of fibrous granulation tissue, which may arise as a consequence of acute pancreatitis, pancreatic trauma or CP[3]. Biliary obstruction may be caused by fibrosis, compression by a PPC or cancer. The present review covers the full spectrum of endoscopic management of local complications of CP; it is not an analysis of specific studies and it does not encompass the management of uncomplicated CP, which has recently been reviewed elsewhere[4].

SEARCHES

Searches for relevant articles were conducted in Medline through PubMed on May 2013, without time limits, using the following search terms: "pancreatitis, chronic"[MeSH Terms] OR ("pancreatitis"[All Fields] AND "chronic" [All Fields]) OR "chronic pancreatitis"[All Fields] OR ("chronic"[All Fields] AND "pancreatitis"[All Fields]) AND pseudocyst[All Fields] AND ("endoscopy"[MeSH Terms] OR "endoscopy"[All Fields]) OR "pancreatitis, chronic"[MeSH Terms] OR ("pancreatitis"[All Fields] AND "chronic"[All Fields]) OR "chronic pancreatitis" [All Fields] OR ("chronic"[All Fields] AND "pancreatitis" [All Fields]) AND pseudocysts[All Fields] AND ("surgery"[Subheading] OR "surgery"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "surgery"[All Fields] OR "general surgery" [MeSH Terms] OR ("general"[All Fields] AND "surgery" [All Fields]) OR "general surgery"[All Fields]); "pancreatitis, chronic"[MeSH Terms] OR ("pancreatitis"[All Fields] AND "chronic"[All Fields]) OR "chronic pancreatitis"[All Fields] OR ("chronic"[All Fields] AND "pancreatitis"[All Fields]) AND biliary[All Fields] AND ("endoscopy"[MeSH Terms] OR "endoscopy"[All Fields]). Articles written in English were selected for complete review on the basis of the abstract. Additional papers were identified by manually checking the reference lists of the articles selected for review.

PANCREATIC PSEUDOCYSTS

Differential diagnosis of pseudocyst-cystic neoplasm

Pseudocysts are the most frequent pancreatic fluid collections. The differential diagnosis between PPCs and cystic neoplasms or, less frequently, necrotized tumors, may be difficult in patients who present for the first time with a pancreatic fluid collection. Amongst the various cystic neoplasms that may affect the pancreas, mucinous cystic neoplasms and intraductal papillary mucinous neoplasms harbor a malignant potential-many of which require surgical resection[5]. As only a few of the available tests provide a high degree of certainty, a diagnosis is usually made by analyzing a set of data, including demographic data and clinical history[6], cross-sectional imaging[7], and endosonography-guided sampling of the fluid content and of the wall of the lesion[8,9]. Research has recently focused on the identification of new biomarkers and on *in vivo* confocal microscopic examination of the cyst wall through a needle inserted under endosonographic guidance[10,11].

Indications for treatment

Widely accepted indications for PPC treatment include the presence of symptoms such as abdominal pain, gastric outlet obstruction, early satiety, weight loss, jaundice, and infected or enlarging PPC[12]. Some authors also recommend treating PPCs in asymptomatic patients to prevent potential PPC-related complications, although these occur only in a minority of patients[13,14]. Other such debated indications include compression of major vessels, intracystic hemorrhage, pancreaticopleural fistula, and PPCs with a diameter greater than 5 cm without any regression after more than 6 wk and a cyst wall thickness larger than 5 mm[15]. In patients with CP, PPCs rarely resolve spontaneously, particularly if their diameter is greater than 4 cm or if they have developed outside of the pancreas[16].

Results: Choosing endoscopic vs surgical treatment

Endoscopic drainage is recommended as a first-line treatment of accessible uncomplicated PPCs because it provides significantly better results compared to surgery in terms of cost, duration of hospital stay and quality of life up to three months post-procedure, as demonstrated in a small randomized controlled trial (RCT)[17]. Reviews of non-comparative historical series of endoscopic and of surgical treatments of PPCs have reported similar results for both modalities in terms of morbidity, with 13% for endoscopic treatments and 16% for surgical treatments. A PPC recurrence rate during long-term follow-up has also been reported of 11% *vs* 10%, respectively, for endoscopic and surgical treatments. An advantage was found in favor of the endoscopic method in terms of mortality (0.2% *vs* 2.5%)[15,18].

Some, but not all, authors have reported that endoscopic PPC drainage yielded higher success rates in the setting of CP *vs* acute pancreatitis. For example, Baron *et al*[19] reported resolution of 92% of chronic pseudocysts *vs* 74% of acute pseudocysts in a series of 138 patients while Hookey *et al*[20] reported resolution of 94% of chronic pseudocysts *vs* 92% of acute pseudocysts in a series of 116 patients.

A first-line surgical approach is usually adopted if necrosis has not yet liquefied and if treatment cannot be delayed. Endoscopy carries a lower success rate and higher morbidity rate in such instances; the reader is referred to a recent review for the comparison of currently available techniques in this particular indication[21]. Pancreatic necrosectomy requires expert endoscopic skill, dedication and adequate patient selection.

Endoscopic technique

Access route: A direct communication between the PPC and the main pancreatic duct (MPD) may be demonstrated in 40%-66% of all PPCs[22]. Such a communication allows drainage of the PPC *via* a stent inserted into the PPC through the papilla ("transpapillary drainage") as opposed to a stent being inserted into the PPC through the digestive wall ("transmural drainage").

No RCT has compared the transpapillary *vs* the transmural drainage route but, in nonrandomized comparative studies, procedure-related morbidity was lower with the transpapillary route (2% *vs* 15%) and long-term success was similar[20,22,23]. The transpapillary route is usually reserved for relatively small (diameter < 5 cm) PPCs located in the head or the body of the pancreas.

"Conventional" endoscopic-guided vs endosonography-guided technique

Endosonography-guided PPC drainage tends to replace the "conventional" endoscopic approach that uses a duodenoscope or, in some cases, a gastroscope. A recent meta-analysis found that the single demonstrated advantage of the endosonography-guided technique is the possibility to drain non-bulging PPCs[24], which represent approximately half of all PPCs[22]. The most important limitations of the endosonography-guided technique reside in the thinner diameter of the working channel of the echoendoscope and in the lower maneuverability of the elevator. While the "conventional" approach is relatively standardized, new material is constantly being tested to make endosonography-guided PPC drainage a single-step, reliable procedure. One of the most recent devices allows puncturing, dilating the puncture tract and inserting two guidewires into the PPC without any device exchange. The device is made of a catheter with two balloons, one to anchor it to inside the PPC and the other one to dilate the puncture tract[25].

Transgastric vs transduodenal transmural route

Some PPCs may be accessed through either the gastric or the duodenal wall. In such cases, the transduodenal route may be preferable as long-term success has been reported more frequently with the transduodenal *vs* the transgastric route (83% *vs* 64%); procedure-related morbidity was 10% with both routes[26]. The difference in long-term success may be related to the longer durability of cystoduodenal compared with cystogastric fistulas (the latter ones typically close a few days after stent removal).

Number and type of stents

Two double pigtail stents are usually inserted for transmural drainage; a naso-cystic catheter may be left in place to rinse the PPC cavity with saline if debris is present. In a large retrospective series, the insertion of a single *vs* multiple stent was independently associated with the failure of endoscopic PPC drainage, defined as severe procedure-related complication or need for another treatment modality[27]. In that series, straight stents were used and they were associated with frequent bleeding (7% of patients, with surgery required in two thirds of them) and stent migration.

Stenting duration

Enterocystic transmural stents should not be retrieved before PPC resolution and not before at least 2 mo of stenting. This recommendation is mostly based on a RCT that allocated 28 patients (including 15 with CP) who had PPC resolution after transmural drainage to either stent maintenance or early stent retrieval; in the latter allocation group, stent retrieval was performed at a median of 2 mo post stent insertion[28]. PPC recurrence was more frequent in the early stent retrieval group (38% *vs* 0%) and, in another, retrospective, series, a stenting duration of 6 wk or less was independently associated with the failure of endoscopic PPC drainage[27].

Procedure-related complications

Reported figures largely vary from center to center with average morbidity rates of 13% and average mortality rates of 0.3%[15,29]. Major complications include hemorrhage, perforation and infection. Most of these can be managed by non-operative means, including endoscopic coagulation, arterial embolization, repeat endoscopic drainage in the case of secondary infection and antibiotics in the case of retroperitoneal perforation. The following measures may help in preventing procedure-related complications:

Secondary infection: Although no data on the efficacy of antibiotic prophylaxis for endoscopic PPC drainage are available, antibiotic administration has been recommended immediately before transmural or transpapillary PPC drainage[30]. The decision whether to continue antibiotics or not after the procedure should be based on drainage adequacy and on the presence or absence of necrosis[12].

Bleeding: Severe bleeding usually arises from dilated arteries or veins. Pseudoaneurysms of the splenic artery may develop in the vicinity of PPCs. Imaging preceding the endoscopic drainage of PPCs should look for pseudoaneurysms and, in the case that one is discovered, have its prophylactic embolization discussed if the transmural route is elected. Extrahepatic portal hypertension develops during the course of CP in 15% or more of patients. It is frequently associated with PPC as well as leading to higher morbidity in patients who undergo pancreatic surgery[31]. The endosonography-guided technique of PPC drainage has been recommended in such patients although it has not been demonstrated to decrease the risk of bleeding[32].

In the case of infected PPC, should the strategy be different?

Primary infection is a rare complication of CP-related PPCs; secondary infection following stent occlusion or endoscopic attempt at draining pancreatic necrosis is more frequent[33].

Infected PPCs present a thick content that may not drain adequately through one or two thin plastic stents. Traditionally, in such cases, more large-bore stents are inserted together with a nasocystic catheter that is used for PPC irrigation. These additional interventions have resulted in similarly high success rates in patients with infected PPCs as compared with those who present uncomplicated PPC[20,34]. As inserting multiple stents plus a nasocystic catheter requires time and may be technically challenging, fully covered self-expandable metal stents (FCSEMSs) seem to be a promising alternative for draining PPCs with a thick content. In a series of 20 patients with an infected PPC that was drained by endosonography-guided FCSEMS transmural insertion alone, clinical success was achieved in 17 patients[35]. The authors suggested that using FCSEMSs rather than plastic stents plus nasocystic drains in patients with infected PPCs may decrease the number of endoscopic procedures, increase the final success rate, and reduce the time required for PPC resolution. FCSEMSs specifically designed for PPC drainage have become available from various manufacturers; they present a short length, a large lumen, and a diabolo shape aimed at preventing stent migration[25,36].

In the case of complete MPD rupture, should the strategy be different?

If complete MPD rupture occurs, the disconnected pancreatic tail may keep secreting pancreatic juice that, in the absence of effective drainage, will lead to prolonged fluid accumulation. Bridging of complete MPD ruptures should be attempted and a combination of transmural PPC drainage plus a transpapillary stent bridging the MPD rupture should be considered[37,38]. The stent should be left in place for a long duration, at least as long as secretin-enhanced magnetic resonance pancreatography demonstrates juice outflow from the disconnected pancreatic tail[39].

BILIARY STRICTURES

Differential diagnosis

It is of paramount importance to reasonably rule out malignancy before embarking on the endoscopic treatment of presumed CP-related biliary strictures, as such a treatment usually lasts for one year and the course of pancreatic cancer is rapid. Particular attention should be paid to patients who present risk factors for pancreatic cancer; these include patients over 50 years of age, female gender, white race, or an absence of pancreatic calcifications and presence of exocrine insufficiency[40,41]. Patients with hereditary pancreatitis present a very high risk of pancreatic cancer.

The accuracy of standard CT scanning and of endosonography for disclosing pancreatic cancer is limited in patients with CP[42,43]. Endosonography, supplemented by fine needle aspiration (FNA) plus biliary endoluminal sampling and assessment of malignancy biomarkers, is part of the standard work-up of a biliary stricture detected in the setting of CP. Other examination modalities such as probe-based endoluminal real-time microscopy are investigational. It should be kept in mind that endosonography-guided FNA is less accurate in the presence than in the absence of CP[41,44], although this decrease in accuracy has been suggested to be confined to a subset of patients who present with obstructive jaundice and a biliary stent[45]. Furthermore, in the community, the accuracy of endosonography-guided FNA for diagnosing pancreatic cancer is likely to be much lower than the 90% figure that is widely reported in the literature (latter reports originate from tertiary centers and use per-protocol analysis)[46,47]. The technical details of the sampling procedure and of the sample processing are extremely important to reach a high diagnostic accuracy; they have recently been reviewed elsewhere for endosonography-guided FNA and for endoluminal biliary sampling[48-50]. Recent improvements in the field of endoluminal biliary sampling include the development of more effective sampling devices and the use of rapid on-site examination for smears as well as for tissue biopsies[48,51,52].

Indications for treatment

Generally accepted indications for the treatment of CP-related biliary strictures include symptoms such as secondary biliary cirrhosis, biliary stones, progression of biliary stricture, and asymptomatic elevation of serum alkaline phosphatase (greater than 2 or 3 times the upper limit of normal values) or of serum bilirubin or both for longer than one month.

Results: Choosing endoscopic vs surgical treatment

Guidelines recently issued by the European Society of Gastrointestinal Endoscopy propose that the choice between endoscopic and surgical treatment should rely on local expertise, loco-regional or systemic patient comorbidities and expected patient compliance with repeat endoscopic procedures[53]. No strong recommendation could be made about the choice between the endoscopic and the surgical approach to CP-related biliary strictures due to the lack of comparative studies. In conditions different from CP, two comparative nonrandomized studies that included 143 patients with biliary strictures related to a traumatism have found that long-term success was similar (77%-83%) with the endoscopic and the surgical approaches[54,55]. However, the endoscopic techniques used in these studies are not current anymore and the endoscopic treatment is more effective in post-traumatic compared with CP-related biliary strictures[29]. Another study has compared the endoscopic *vs* the surgical drainage of CP-related biliary strictures; however, surgery was performed in only 6 patients, of whom five also had a pancreatic resection[56].

Patient complications such as portal cavernoma or cirrhosis are often decisive factors in the selection of the endoscopic *vs* the surgical modality. Other factors that may influence this selection include the expected patient compliance with endoscopic stent exchanges and, less importantly, the presence or absence of pancreatic calcifications. In a retrospective series of 14 patients, only two patients presented for elective stent exchanges scheduled at 3-mo intervals[57]. Most patients were admitted with biliary infection due to stent occlusion after the scheduled stent exchange date. Another series that included 29 patients treated with multiple, side-by-side, plastic biliary stents reported the occurrence of at least 20 episodes of cholangitis (in this latter series, stents were exchanged when symptoms of clogging developed). The mean interval between stent exchanges was 6 mo in patients who were alive at the end of follow-up as compared to 22 mo ($P < 0.05$) in the three patients who died during follow-up (two of them from cholangitis)[58]. The presence of pancreatic calcifications has been associated with long-term failure of single plastic biliary stenting[59] but this factor may be less relevant if simultaneous multiple, side-by-side, plastic stents are used[60].

Endoscopic technique

Plastic stents: If the endoscopic treatment modality is selected, temporary placement of simultaneous multiple, side-by-side, plastic stents is the gold standard amongst the various techniques available. A single nonrandomized series has compared long-term results after temporary placement of single *vs* multiple simultaneous plastic stents; clinical success was reported in 24% *vs* 92% of patients, respectively[60].

From a practical point of view, amongst plastic biliary stents, polyethylene models are recommended because they allow obstruction relief more frequently than Teflon models[61], and the exchange of plastic stents with an increasing number of stents is usually scheduled at 3-mo intervals for a total stenting duration of 12 mo[58,60,62]. It has recently been suggested that with multiple, side-by-side, plastic stents, the interval between stent exchanges could be extended[63]. However, as mentioned above, special care should be taken regarding the generally poor compliance and poor physical status of patients with alcoholic CP. It is recommended to implement a recall system to care for patients who do not turn up for stent exchanges at scheduled dates.

Self-expandable metal stents: "Definitive" insertion of self-expandable metal stents (SEMSs) (*i.e.*, with no intended SEMS removal) for benign biliary strictures has almost been abandoned due to the development of biliary epithelial hyperplasia that leads to late biliary obstruction[64]. Recent studies of the endoscopic treatment of CP-related biliary strictures have focused on the temporary placement of covered SEMS with a shift of interest from partially covered to fully covered SEMS designs[65,66]. Spontaneous SEMS migration has been the main drawback with FCSEMSs; new stent designs aiming to prevent migration include the adjunction of anchoring fins, the positioning of the stent covering on the internal side of the SEMS and a flared-ends design. Such SEMSs remain investigational for the treatment of benign biliary strictures.

If FCSEMSs are used to treat benign biliary strictures, a stenting duration > 90 d is recommended as this was independently associated with stricture resolution in a multicenter trial that included 133 patients with benign biliary strictures, 44 of these being CP-related[67]. In this trial, stricture resolution at the time of stent removal was reported in 26 (59%, intention-to-treat analysis) patients with CP. Other studies that included more than 10 patients followed up for at least one year after FCSEMS removal showed a success rate of approximately 50%: (1) Perri *et al*[66] inserted a Niti-S stent with either a straight or a flared-ends design in 17 patients who had previously received a single plastic stent. Two years following FCSEMS removal, 56% of patients had presented no stricture relapse and had normal liver function tests. The flared-ends design partially prevented FCSEMS migration while all straight FCSEMSs migrated; and (2) Poley *et al*[68] inserted a Hanaro prototype FCSEMS in 13 patients who had previously received a single plastic stent; success was reported in 6 (43%) of them.

FCSEMS currently are the most promising alternative to multiple, side-by-side, plastic biliary stents. Despite their main advantages, *i.e.*, a reduced number of endoscopy procedures and a lower incidence of stent obstruction, FCSEMSs need improvements in their design as well as additional large multicenter trials before they can possibly be recommended as a first-line option for the endoscopic treatment of CP-related biliary strictures.

OTHER COMPLICATIONS

Other complications of CP include splenic vein thrombosis, pancreatic adenocarcinoma, pancreatic ascites and pleural effusion.

Splenic vein thrombosis is present in approximately 12% of patients with CP and it is usually asymptomatic but may cause bleeding in 7% of patients[69]. In the case of bleeding, endoscopic variceal obturation using *N*-butylcyanoacrylate is effective in achieving hemostasis, and a splenectomy is an effective way to prevent recurrent bleeding[70].

For the treatment of pancreatic adenocarcinoma, the endosonography-guided delivery of various cytotoxic agents is a rapidly evolving field that remains investigational[71].

Pancreatic ascites and pleural effusion are rare complications of CP; they may or may not be associated with PPC and they present a high morbidity. The aim of endoscopic therapy in such patients is to insert a stent to bridge the MPD rupture that is responsible for pancreatic juice leakage; high success rates have been reported[72].

CONCLUSION

Progress has recently been made in the field of the endoscopic treatment of CP-related complications.

With regard to uncomplicated PPCs, endoscopic drainage has been shown to be feasible in almost all cases and to be superior to surgical drainage. Techniques associated with the best clinical outcome have been identified. With regard to CP-related biliary obstructions, improvements in the design of FCSEMSs are challenging the standard technique and have the potential to improve patient acceptability.

REFERENCES

1 **Abdallah AA**, Krige JE, Bornman PC. Biliary tract obstruction in chronic pancreatitis. *HPB (Oxford)* 2007; **9**: 421-428 [PMID: 18345288 DOI: 10.1080/13651820701774883]

2 **Andrén-Sandberg A**, Dervenis C. Pancreatic pseudocysts in the 21st century. Part I: classification, pathophysiology, anatomic considerations and treatment. *JOP* 2004; **5**: 8-24 [PMID: 14730118]

3 **Bradley EL**. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993; **128**: 586-590 [PMID: 8489394]

4 **Dumonceau JM**. Endoscopic management of chronic pancreatitis: an evidence-based approach. In: Testoni P, Mariani A, Arcidiacono PG. Acute and chronic pancreatitis: new concepts and evidence-based approaches. Testoni P, editor. Milan: Minerva Medica, 2013: 103-108

5 **Tanaka M**, Fernández-del Castillo C, Adsay V, Chari S, Falconi M, Jang JY, Kimura W, Levy P, Pitman MB, Schmidt CM, Shimizu M, Wolfgang CL, Yamaguchi K, Yamao K. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. *Pancreatology* 2012: 183-197 [PMID: 22687371 DOI: 10.1016/j.pan.2012.04.004]

6 **Talamini G**, Zamboni G, Salvia R, Capelli P, Sartori N, Casetti L, Bovo P, Vaona B, Falconi M, Bassi C, Scarpa A, Vantini I, Pederzoli P. Intraductal papillary mucinous neoplasms and chronic pancreatitis. *Pancreatology* 2006; **6**: 626-634 [PMID: 17135772 DOI: 10.1159/000097605]

7 **Chalian H**, Töre HG, Miller FH, Yaghmai V. CT attenuation of unilocular pancreatic cystic lesions to differentiate pseudocysts from mucin-containing cysts. *JOP* 2011; **12**: 384-388 [PMID: 21737901]

8 **Brugge WR**, Lauwers GY, Sahani D, Fernandez-del Castillo C, Warshaw AL. Cystic neoplasms of the pancreas. *N Engl J Med* 2004; **351**: 1218-1226 [PMID: 15371579 DOI: 10.1056/NEJMra031623]

9 **van der Waaij LA**, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. *Gastrointest Endosc* 2005; **62**: 383-389 [PMID: 16111956]

10 **Cuoghi A**, Farina A, Z'graggen K, Dumonceau JM, Tomasi A, Hochstrasser DF, Genevay M, Lescuyer P, Frossard JL. Role of proteomics to differentiate between benign and potentially malignant pancreatic cysts. *J Proteome Res* 2011; **10**: 2664-2670 [PMID: 21425880 DOI: 10.1021/pr2000557]

11 **Konda VJ**, Aslanian HR, Wallace MB, Siddiqui UD, Hart J, Waxman I. First assessment of needle-based confocal laser endomicroscopy during EUS-FNA procedures of the pancreas (with videos). *Gastrointest Endosc* 2011; **74**: 1049-1060 [PMID: 21924718 DOI: 10.1016/j.gie.2011.07.018]

12 **Jacobson BC**, Baron TH, Adler DG, Davila RE, Egan J, Hirota WK, Leighton JA, Qureshi W, Rajan E, Zuckerman MJ, Fanelli R, Wheeler-Harbaugh J, Faigel DO. ASGE guideline: The role of endoscopy in the diagnosis and the management of cystic lesions and inflammatory fluid collections of the pancreas. *Gastrointest Endosc* 2005; **61**: 363-370 [PMID: 15758904]

13 **Vitas GJ**, Sarr MG. Selected management of pancreatic pseudocysts: operative versus expectant management. *Surgery* 1992; **111**: 123-130 [PMID: 1736380]

14 **Yeo CJ**, Bastidas JA, Lynch-Nyhan A, Fishman EK, Zinner MJ, Cameron JL. The natural history of pancreatic pseudocysts documented by computed tomography. *Surg Gynecol Obstet* 1990; **170**: 411-417 [PMID: 2326721]

15 **Lerch MM**, Stier A, Wahnschaffe U, Mayerle J. Pancreatic pseudocysts: observation, endoscopic drainage, or resection? *Dtsch Arztebl Int* 2009; **106**: 614-621 [PMID: 19890418 DOI: 10.3238/arztebl.2009.0614]

16 **Gouyon B**, Lévy P, Ruszniewski P, Zins M, Hammel P, Vilgrain V, Sauvanet A, Belghiti J, Bernades P. Predictive factors in the outcome of pseudocysts complicating alcoholic chronic pancreatitis. *Gut* 1997; **41**: 821-825 [PMID: 9462217]

17 **Varadarajulu S**, Bang JY, Sutton BS, Trevino JM, Christein JD, Wilcox CM. Equal efficacy of endoscopic and surgical cystogastrostomy for pancreatic pseudocyst drainage in a randomized trial. *Gastroenterology* 2013; **145**: 583-590.e1 [PMID: 23732774 DOI: 10.1053/j.gastro.2013.05.046]

18 **Rosso E**, Alexakis N, Ghaneh P, Lombard M, Smart HL, Evans J, Neoptolemos JP. Pancreatic pseudocyst in chronic pancreatitis: endoscopic and surgical treatment. *Dig Surg* 2003; **20**: 397-406 [PMID: 12900529 DOI: 10.1159/000072706]

19 **Baron TH**, Harewood GC, Morgan DE, Yates MR. Outcome differences after endoscopic drainage of pancreatic necrosis, acute pancreatic pseudocysts, and chronic pancreatic pseudocysts. *Gastrointest Endosc* 2002; **56**: 7-17 [PMID: 12085029]

20 **Hookey LC**, Debroux S, Delhaye M, Arvanitakis M, Le Moine O, Devière J. Endoscopic drainage of pancreatic-fluid collections in 116 patients: a comparison of etiologies, drainage techniques, and outcomes. *Gastrointest Endosc* 2006; **63**: 635-643 [PMID: 16564865 DOI: 10.1016/j.gie.2005.06.028]

21 **Bello B**, Matthews JB. Minimally invasive treatment of pancreatic necrosis. *World J Gastroenterol* 2012; **18**: 6829-6835 [PMID: 23239921 DOI: 10.3748/wjg.v18.i46.6829]

22 **Barthet M**, Lamblin G, Gasmi M, Vitton V, Desjeux A, Grimaud JC. Clinical usefulness of a treatment algorithm for pancreatic pseudocysts. *Gastrointest Endosc* 2008; **67**: 245-252 [PMID: 18226686 DOI: 10.1016/j.gie.2007.06.014]

23 **Binmoeller KF**, Seifert H, Walter A, Soehendra N. Transpapillary and transmural drainage of pancreatic pseudocysts. *Gastrointest Endosc* 1995; **42**: 219-224 [PMID: 7498686]

24 **Panamonta N**, Ngamruengphong S, Kijsirichareanchai K, Nugent K, Rakvit A. Endoscopic ultrasound-guided versus conventional transmural techniques have comparable treatment outcomes in draining pancreatic pseudocysts. *Eur J Gastroenterol Hepatol* 2012; **24**: 1355-1362 [PMID: 23114741 DOI: 10.1097/MEG.0b013e32835871eb]

25 **Binmoeller KF**, Weilert F, Shah JN, Bhat YM, Kane S. Endosonography-guided transmural drainage of pancreatic pseudocysts using an exchange-free access device: initial clinical experience. *Surg Endosc* 2013; **27**: 1835-1839 [PMID: 23299130 DOI: 10.1007/s00464-012-2682-9]

26 **Beckingham IJ**, Krige JE, Bornman PC, Terblanche J. Endoscopic management of pancreatic pseudocysts. *Br J Surg* 1997; **84**: 1638-1645 [PMID: 9448608]

27 **Cahen D**, Rauws E, Fockens P, Weverling G, Huibregtse K, Bruno M. Endoscopic drainage of pancreatic pseudocysts: long-term outcome and procedural factors associated with safe and successful treatment. *Endoscopy* 2005; **37**: 977-983 [PMID: 16189770 DOI: 10.1055/s-2005-870336]

28 **Arvanitakis M**, Delhaye M, Bali MA, Matos C, De Maertelaer V, Le Moine O, Devière J. Pancreatic-fluid collections: a randomized controlled trial regarding stent removal after endoscopic transmural drainage. *Gastrointest Endosc* 2007; **65**: 609-619 [PMID: 17324413 DOI: 10.1016/j.gie.2006.06.083]

29 **Nguyen-Tang T**, Dumonceau JM. Endoscopic treatment in chronic pancreatitis, timing, duration and type of intervention. *Best Pract Res Clin Gastroenterol* 2010; **24**: 281-298 [PMID:

20510829 DOI: 10.1016/j.bpg.2010.03.002]

30 **Banerjee S**, Shen B, Baron TH, Nelson DB, Anderson MA, Cash BD, Dominitz JA, Gan SI, Harrison ME, Ikenberry SO, Jagannath SB, Lichtenstein D, Fanelli RD, Lee K, van Guilder T, Stewart LE. Antibiotic prophylaxis for GI endoscopy. *Gastrointest Endosc* 2008; **67**: 791-798 [PMID: 18374919 DOI: 10.1016/j.gie.2008.02.068]

31 **Izbicki JR**, Yekebas EF, Strate T, Eisenberger CF, Hosch SB, Steffani K, Knoefel WT. Extrahepatic portal hypertension in chronic pancreatitis: an old problem revisited. *Ann Surg* 2002; **236**: 82-89 [PMID: 12131089]

32 **Sriram PV**, Kaffes AJ, Rao GV, Reddy DN. Endoscopic ultrasound-guided drainage of pancreatic pseudocysts complicated by portal hypertension or by intervening vessels. *Endoscopy* 2005; **37**: 231-235 [PMID: 15731938 DOI: 10.1055/s-2005-860997]

33 **Andrén-Sandberg A**, Dervenis C. Pancreatic pseudocysts in the 21st century. Part II: natural history. *JOP* 2004; **5**: 64-70 [PMID: 15007187]

34 **Varadarajulu S**, Bang JY, Phadnis MA, Christein JD, Wilcox CM. Endoscopic transmural drainage of peripancreatic fluid collections: outcomes and predictors of treatment success in 211 consecutive patients. *J Gastrointest Surg* 2011; **15**: 2080-2088 [PMID: 21786063 DOI: 10.1007/s11605-011-1621-8]

35 **Fabbri C**, Luigiano C, Cennamo V, Polifemo AM, Barresi L, Jovine E, Traina M, D'Imperio N, Tarantino I. Endoscopic ultrasound-guided transmural drainage of infected pancreatic fluid collections with placement of covered self-expanding metal stents: a case series. *Endoscopy* 2012; **44**: 429-433 [PMID: 22382852 DOI: 10.1055/s-0031-1291624]

36 **Itoi T**, Binmoeller KF, Shah J, Sofuni A, Itokawa F, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Moriyasu F. Clinical evaluation of a novel lumen-apposing metal stent for endosonography-guided pancreatic pseudocyst and gallbladder drainage (with videos). *Gastrointest Endosc* 2012; **75**: 870-876 [PMID: 22301347 DOI: 10.1016/j.gie.2011.10.020]

37 **Pelaez-Luna M**, Vege SS, Petersen BT, Chari ST, Clain JE, Levy MJ, Pearson RK, Topazian MD, Farnell MB, Kendrick ML, Baron TH. Disconnected pancreatic duct syndrome in severe acute pancreatitis: clinical and imaging characteristics and outcomes in a cohort of 31 cases. *Gastrointest Endosc* 2008; **68**: 91-97 [PMID: 18378234 DOI: 10.1016/j.gie.2007.11.041]

38 **Trevino JM**, Tamhane A, Varadarajulu S. Successful stenting in ductal disruption favorably impacts treatment outcomes in patients undergoing transmural drainage of peripancreatic fluid collections. *J Gastroenterol Hepatol* 2010; **25**: 526-531 [PMID: 20074158 DOI: 10.1111/j.1440-1746.2009.06109.x]

39 **Varadarajulu S**, Noone TC, Tutuian R, Hawes RH, Cotton PB. Predictors of outcome in pancreatic duct disruption managed by endoscopic transpapillary stent placement. *Gastrointest Endosc* 2005; **61**: 568-575 [PMID: 15812410]

40 **Arvanitakis M**, Van Laethem JL, Parma J, De Maertelaer V, Delhaye M, Devière J. Predictive factors for pancreatic cancer in patients with chronic pancreatitis in association with K-ras gene mutation. *Endoscopy* 2004; **36**: 535-542 [PMID: 15202051 DOI: 10.1055/s-2004-814401]

41 **Varadarajulu S**, Tamhane A, Eloubeidi MA. Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. *Gastrointest Endosc* 2005; **62**: 728-36; quiz 751, 753 [PMID: 16246688 DOI: 10.1016/j.gie.2005.06.051]

42 **Ardengh JC**, Lopes CV, Campos AD, Pereira de Lima LF, Venco F, Módena JL. Endoscopic ultrasound and fine needle aspiration in chronic pancreatitis: differential diagnosis between pseudotumoral masses and pancreatic cancer. *JOP* 2007; **8**: 413-421 [PMID: 17625292]

43 **Bipat S**, Phoa SS, van Delden OM, Bossuyt PM, Gouma DJ, Laméris JS, Stoker J. Ultrasonography, computed tomography and magnetic resonance imaging for diagnosis and determining resectability of pancreatic adenocarcinoma: a meta-analysis. *J Comput Assist Tomogr* 2005: 438-445 [PMID: 16012297]

44 **Fritscher-Ravens A**, Brand L, Knöfel WT, Bobrowski C, Topalidis T, Thonke F, de Werth A, Soehendra N. Comparison of endoscopic ultrasound-guided fine needle aspiration for focal pancreatic lesions in patients with normal parenchyma and chronic pancreatitis. *Am J Gastroenterol* 2002; **97**: 2768-2775 [PMID: 12425546 DOI: 10.1111/j.1572-0241.2002.07020.x]

45 **Krishna NB**, Mehra M, Reddy AV, Agarwal B. EUS/EUS-FNA for suspected pancreatic cancer: influence of chronic pancreatitis and clinical presentation with or without obstructive jaundice on performance characteristics. *Gastrointest Endosc* 2009; **70**: 70-79 [PMID: 19249774 DOI: 10.1016/j.gie.2008.10.030]

46 **Dumonceau JM**, Koessler T, van Hooft JE, Fockens P. Endoscopic ultrasonography-guided fine needle aspiration: Relatively low sensitivity in the endosonographer population. *World J Gastroenterol* 2012; **18**: 2357-2363 [PMID: 22654426 DOI: 10.3748/wjg.v18.i19.2357]

47 **Rösch T**, Hofrichter K, Frimberger E, Meining A, Born P, Weigert N, Allescher HD, Classen M, Barbur M, Schenck U, Werner M. ERCP or EUS for tissue diagnosis of biliary strictures? A prospective comparative study. *Gastrointest Endosc* 2004; **60**: 390-396 [PMID: 15332029]

48 **Dumonceau JM**, Polkowski M, Larghi A, Vilmann P, Giovannini M, Frossard JL, Heresbach D, Pujol B, Fernández-Esparrach G, Vazquez-Sequeiros E, Ginès A. Indications, results, and clinical impact of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2011; **43**: 897-912 [PMID: 21842456 DOI: 10.1055/s-0030-1256754]

49 **Polkowski M**, Larghi A, Weynand B, Boustière C, Giovannini M, Pujol B, Dumonceau JM. Learning, techniques, and complications of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline. *Endoscopy* 2012; **44**: 190-206 [PMID: 22180307 DOI: 10.1055/s-0031-1291543]

50 **Dumonceau JM**. Sampling at ERCP for cyto- and histopathologicical examination. *Gastrointest Endosc Clin N Am* 2012; **22**: 461-477 [PMID: 22748243 DOI: 10.1016/j.giec.2012.05.006]

51 **Wright ER**, Bakis G, Srinivasan R, Raju R, Vittal H, Sanders MK, Bernadino K, Stefan A, Blaszyk H, Howell DA. Intraprocedural tissue diagnosis during ERCP employing a new cytology preparation of forceps biopsy (Smash protocol). *Am J Gastroenterol* 2011; **106**: 294-299 [PMID: 21102569 DOI: 10.1038/ajg.2010.447]

52 **Kawada N**, Uehara H, Katayama K, Takano Y, Takakura R, Imanaka K, Ohkawa K, Takenaka A, Ishikawa O. Combined brush cytology and stent placement in a single session for presumed malignant biliary stricture. *J Gastroenterol Hepatol* 2011; **26**: 1247-1251 [PMID: 21443662 DOI: 10.1111/j.1440-1746.2011.06725.x]

53 **Dumonceau JM**, Delhaye M, Tringali A, Dominguez-Munoz JE, Poley JW, Arvanitaki M, Costamagna G, Costea F, Devière J, Eisendrath P, Lakhtakia S, Reddy N, Fockens P, Ponchon T, Bruno M. Endoscopic treatment of chronic pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2012; **44**: 784-800 [PMID: 22752888 DOI: 10.1055/s-0032-1309840]

54 **Davids PH**, Tanka AK, Rauws EA, van Gulik TM, van Leeuwen DJ, de Wit LT, Verbeek PC, Huibregtse K, van der Heyde MN, Tytgat GN. Benign biliary strictures. Surgery or endoscopy? *Ann Surg* 1993; **217**: 237-243 [PMID: 8452402]

55 **Tocchi A**, Mazzoni G, Liotta G, Costa G, Lepre L, Miccini M, De Masi E, Lamazza MA, Fiori E. Management of benign biliary strictures: biliary enteric anastomosis vs endoscopic stenting. *Arch Surg* 2000; **135**: 153-157 [PMID: 10668872]

56 **Regimbeau JM**, Fuks D, Bartoli E, Fumery M, Hanes A, Yzet

T, Delcenserie R. A comparative study of surgery and endoscopy for the treatment of bile duct stricture in patients with chronic pancreatitis. *Surg Endosc* 2012; **26**: 2902-2908 [PMID: 22580872 DOI: 10.1007/s00464-012-2283-7]

57 **Kiehne K**, Fölsch UR, Nitsche R. High complication rate of bile duct stents in patients with chronic alcoholic pancreatitis due to noncompliance. *Endoscopy* 2000; **32**: 377-380 [PMID: 10817175]

58 **Pozsár J**, Sahin P, László F, Forró G, Topa L. Medium-term results of endoscopic treatment of common bile duct strictures in chronic calcifying pancreatitis with increasing numbers of stents. *J Clin Gastroenterol* 2004; **38**: 118-123 [PMID: 14745285]

59 **Kahl S**, Zimmermann S, Genz I, Glasbrenner B, Pross M, Schulz HU, Mc Namara D, Schmidt U, Malfertheiner P. Risk factors for failure of endoscopic stenting of biliary strictures in chronic pancreatitis: a prospective follow-up study. *Am J Gastroenterol* 2003; **98**: 2448-2453 [PMID: 14638347]

60 **Catalano MF**, Linder JD, George S, Alcocer E, Geenen JE. Treatment of symptomatic distal common bile duct stenosis secondary to chronic pancreatitis: comparison of single vs. multiple simultaneous stents. *Gastrointest Endosc* 2004; **60**: 945-952 [PMID: 15605010]

61 **Dumonceau JM**, Heresbach D, Devière J, Costamagna G, Beilenhoff U, Riphaus A. Biliary stents: models and methods for endoscopic stenting. *Endoscopy* 2011; **43**: 617-626 [PMID: 21614754 DOI: 10.1055/s-0030-1256315]

62 **Draganov P**, Hoffman B, Marsh W, Cotton P, Cunningham J. Long-term outcome in patients with benign biliary strictures treated endoscopically with multiple stents. *Gastrointest Endosc* 2002; **55**: 680-686 [PMID: 11979250]

63 **Lawrence C**, Romagnuolo J, Payne KM, Hawes RH, Cotton PB. Low symptomatic premature stent occlusion of multiple plastic stents for benign biliary strictures: comparing standard and prolonged stent change intervals. *Gastrointest Endosc* 2010; **72**: 558-563 [PMID: 20638060 DOI: 10.1016/j.gie.2010.05.029]

64 **Dumonceau JM**, Tringali A, Blero D, Devière J, Laugiers R, Heresbach D, Costamagna G. Biliary stenting: indications, choice of stents and results: European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. *Endoscopy* 2012; **44**: 277-298 [PMID: 22297801 DOI: 10.1055/s-0031-1291633]

65 **Behm B**, Brock A, Clarke BW, Ellen K, Northup PG, Dumonceau JM, Kahaleh M. Partially covered self-expandable metallic stents for benign biliary strictures due to chronic pancreatitis. *Endoscopy* 2009; **41**: 547-551 [PMID: 19533560 DOI: 10.1055/s-0029-1214708]

66 **Perri V**, Boškoski I, Tringali A, Familiari P, Mutignani M, Marmo R, Costamagna G. Fully covered self-expandable metal stents in biliary strictures caused by chronic pancreatitis not responding to plastic stenting: a prospective study with 2 years of follow-up. *Gastrointest Endosc* 2012; **75**: 1271-1277 [PMID: 22464813 DOI: 10.1016/j.gie.2012.02.002]

67 **Kahaleh M**, Brijbassie A, Sethi A, Degaetani M, Poneros JM, Loren DE, Kowalski TE, Sejpal DV, Patel S, Rosenkranz L, McNamara KN, Raijman I, Talreja JP, Gaidhane M, Sauer BG, Stevens PD. Multicenter Trial Evaluating the Use of Covered Self-expanding Metal Stents in Benign Biliary Strictures: Time to Revisit Our Therapeutic Options? *J Clin Gastroenterol* 2013; **47**: 695-699 [PMID: 23442836 DOI: 10.1097/MCG.0b013e31827fd311]

68 **Poley JW**, Cahen DL, Metselaar HJ, van Buuren HR, Kazemier G, van Eijck CH, Haringsma J, Kuipers EJ, Bruno MJ. A prospective group sequential study evaluating a new type of fully covered self-expandable metal stent for the treatment of benign biliary strictures (with video). *Gastrointest Endosc* 2012; **75**: 783-789 [PMID: 22325806 DOI: 10.1016/j.gie.2011.10.022]

69 **Butler JR**, Eckert GJ, Zyromski NJ, Leonardi MJ, Lillemoe KD, Howard TJ. Natural history of pancreatitis-induced splenic vein thrombosis: a systematic review and meta-analysis of its incidence and rate of gastrointestinal bleeding. *HPB (Oxford)* 2011; **13**: 839-845 [PMID: 22081918 DOI: 10.1111/j.1477-2574.2011.00375.x]

70 **Kang EJ**, Jeong SW, Jang JY, Cho JY, Lee SH, Kim HG, Kim SG, Kim YS, Cheon YK, Cho YD, Kim HS, Kim BS. Long-term result of endoscopic Histoacryl (N-butyl-2-cyanoacrylate) injection for treatment of gastric varices. *World J Gastroenterol* 2011; **17**: 1494-1500 [PMID: 21472110 DOI: 10.3748/wjg.v17.i11.1494]

71 **Carrara S**, Petrone MC, Testoni PA, Arcidiacono PG. Tumors and new endoscopic ultrasound-guided therapies. *World J Gastrointest Endosc* 2013; **5**: 141-147 [PMID: 23596535 DOI: 10.4253/wjge.v5.i4.141]

72 **Pai CG**, Suvarna D, Bhat G. Endoscopic treatment as first-line therapy for pancreatic ascites and pleural effusion. *J Gastroenterol Hepatol* 2009; **24**: 1198-1202 [PMID: 19486258 DOI: 10.1111/j.1440-1746.2009.05796.x]

P- Reviewers: Bramhall SR, Chowdhury P, Montalto G, Maluf F, Tandon RK **S- Editor**: Gou SX **L- Editor**: Logan S **E- Editor**: Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7316

World J Gastroenterol 2013 November 14; 19(42): 7316-7326
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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REVIEW

Multimodal treatment of hepatocellular carcinoma on cirrhosis: An update

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Received: July 3, 2013 **Revised:** August 8, 2013
Accepted: September 16, 2013
Published online: November 14, 2013

Abstract

Hepatocellular carcinoma (HCC) is the most frequent primary liver tumor, and overall, it is one of the most frequent cancers. The association of HCC with chronic liver disease, and cirrhosis in particular, is well known, making treatment complex and challenging. The treatment of HCC must take into account the presence and stage of chronic liver disease, with the aim of preserving hepatic function that is often already impaired, the stage of HCC and the clinical condition of the patient. The different treatment options include surgical resection, transplantation, local ablation, chemoembolization, radioembolization and molecular targeted therapies; these treatments can be combined in various ways to achieve different goals. Ideally, liver transplantation is best treatment for early stage HCC on cirrhosis because it removes both the tumor and the chronic disease that produced it; however, the application of this powerful tool is limited by the scarcity of donors. Downstaging and bridging are different strategies for the management of HCC patients who will undergo liver transplantation. Several professionals, including gastroenterologists, radiologists and surgeons, are involved in the choice of the most appropriate treatment for a single case, and a multidisciplinary approach is necessary to optimize the outcome. The purpose of this review is to provide a comprehensive description of the current treatment options for patients with HCC by analyzing the advantages, disadvantages and rationale for their use.

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Key words: Hepatocellular carcinoma; Multimodal treatment; Locoregional treatments; Molecular targeted therapies; Liver resection; Liver transplantation

Core tip: Hepatocellular carcinoma (HCC) occurs frequently, and its association with cirrhosis makes treatment complex and challenging. The treatment of HCC must take into account the presence and stage of chronic liver disease with the aim of preserving hepatic function that is often already impaired. The different treatment options include surgical resection, transplantation, local ablation, chemoembolization, radioembolization and molecular targeted therapies. Downstaging and bridging are different strategies for the management of HCC patients who will undergo liver transplantation. The purpose of this review is to provide a comprehensive description of the current treatment options for patients with HCC.

Vivarelli M, Montalti R, Risaliti A. Multimodal treatment of hepatocellular carcinoma on cirrhosis: An update. *World J Gastroenterol* 2013; 19(42): 7316-7326 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7316.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7316

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for nearly

90% of the primary liver tumors and is currently the third leading cause of cancer death worldwide. Approximately 500000 new cases of HCC are diagnosed worldwide each year, with a peak incidence observed in countries in which the hepatitis B virus (HBV) is endemic, such as Southeast Asia and sub-Saharan Africa[1]. As the number of carriers of chronic liver disease increases, HCC has become a major public health issue.

The main risk factor for HCC is the presence of chronic liver disease, particularly when the disease has already resulted in liver cirrhosis. The constant process of destruction and repair within the parenchyma that is associated with cirrhosis increases hepatocyte metabolism and amplifies the risk of mutations in a multistep progression from hyperplastic nodule to early HCC and finally to moderately/poorly differentiated HCC.

Hepatitis B and C viruses (HBV and HCV) are known to have oncogenic potential, and the risk of HCC in HBV and HCV carriers is increased independently of the presence of cirrhosis[2,3]. Malignant transformation of hepatocytes in the infected liver could be caused by chronic inflammation and the oxidative DNA damage that leads to genetic and epigenetic changes. There is also evidence that proteins encoded by HBV and HCV may have a direct role in hepatocarcinogenesis; in fact, proteins encoded in the genome of each of these viruses have been linked to alterations in hepatocyte physiology and hepatocellular signal transduction[4]. Other causes of chronic liver diseases, such as alcohol abuse, non-alcoholic steatohepatitis (NASH), hemochromatosis, α1-antitrypsin deficiency, autoimmune disease and Wilson disease are associated with a higher risk of developing HCC and require close patient monitoring. In Western countries, obesity and metabolic syndromes associated with type Ⅱ diabetes, which are strictly related with NASH, are now emerging as new potential predisposing factors for HCC[1].

Among patients with cirrhosis, the cumulative 5-year risk of developing HCC ranges from 5% to 30%, depending on the presence and stage of underlying liver disease, ethnicity, age, sex and duration of the exposure to primary hepatotropic viruses[1,5].

The main peculiarity of HCC is that the treatment of the tumor must take into account the presence and stage of chronic liver disease, with the aim of preserving hepatic function that is often already impaired. A key step in the choice of therapy is therefore the correct assessment of the functional reserve of the liver, which is often more important than the staging of the tumor itself.

Several options are available for the treatment of HCC, and these can often be combined; the choice of treatment and the timing of its administration therefore must be balanced accurately.

The aim of the present review is to provide an update than can be useful in clinical practice for determining the most appropriate treatment for HCC patients.

HCC SCREENING

The achievement of a curative treatment for HCC depends on the detection of the tumor at an early stage. Once the population at risk is identified, screening of HCC is based on ultrasonography and measurement of serum alpha-fetoprotein (s-AFP) levels.

It is recommended that patients with advanced liver fibrosis (F3) or established cirrhosis undergo a liver ultrasound (US) and serum measurements every 6 mo[6]. In these patients, ultrasound has a sensitivity between 58% and 89% and a specificity of 90%[7]. The sensitivity of s-AFP measurements is lower and ranges between 25% and 65% when values above 20 ng/mL are considered positive[1]. The sensitivity and specificity of s-AFP measurement for the detection of HCC increase proportionally with higher blood s-AFP levels, particularly when the level is above 400 ng/mL.

Once the presence of HCC is confirmed, the s-AFP level can be correlated with tumor stage, particularly with the size and multifocality of the tumor and the presence of microvascular invasion. Overall, a screening strategy that combines abdominal ultrasonography and measurement of s-AFP every 6 mo in patients with cirrhosis can reduce HCC mortality by approximately 40%[8,9].

HCC DIAGNOSIS

The cirrhotic liver often displays multiple non-neoplastic nodules (regenerative nodules) that result from the chronic inflammatory process. These nodules must be differentiated from HCC, and the diagnostic strategy varies depending on the size of the nodule detected on ultrasound. At present, the most widely used diagnostic algorithm for the diagnosis of HCC is that proposed by the American Association for the Study of Liver Diseases (AASLD)[10] (Figure 1, modified by Forner *et al*[11]).

The nature of nodules with a diameter of less than 1 cm cannot be precisely defined during ultrasound, and a follow-up control after 3-4 mo is required. Nodules detected at US with a diameter greater than 10 mm must be further investigated with contrast-enhanced triphasic or quadriphasic computed tomography (CT) imaging or magnetic resonance (MR) imaging. The diagnosis of HCC is based on an arterial hypervascular phase (wash-in) followed by disappearance of the contrast in the venous phase (wash-out)[12].

Recent reports have demonstrated that MR has a higher sensitivity compared with CT[13]; however, if the data from the first imaging procedure are not conclusive, confirmation using a different technique is recommended. In cases in which the diagnosis is uncertain, a s-AFP level > 400 ng/mL has a high positive predictive value[1]. Histological confirmation through percutaneous liver biopsy should be restricted to those nodules with features on MR or CT that are not typical enough to allow a diagnosis[14]. In fact, the histological diagnosis of HCC is complex, requires a great degree of expertise and relies on the assumption that the core of the nodule has been effectively sampled by the small needle that is used for the percutaneous biopsy. The sensitivity and specificity reported for nodules less than 20 mm in diameter is

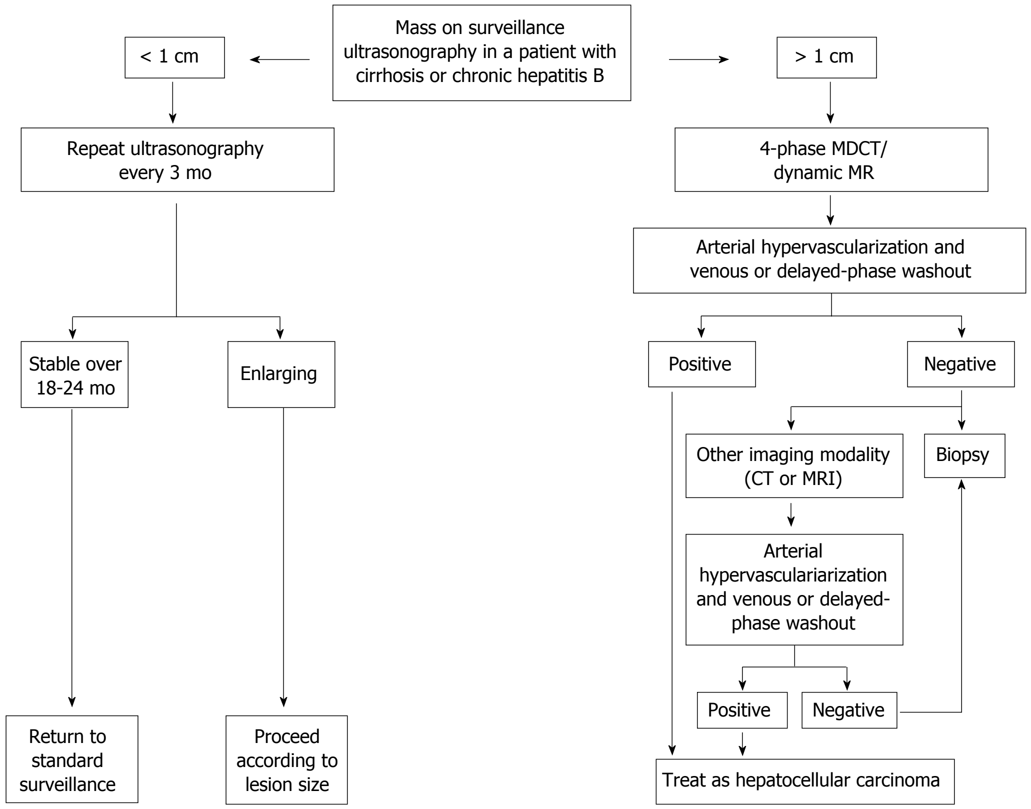
Mass on surveillance ultrasonography in a patient with cirrhosis or chronic hepatitis B
< 1 cm
> 1 cm
Repeat ultrasonography every 3 mo
4-phase MDCT/ dynamic MR
Arterial hypervascularization and venous or delayed-phase washout
Stable over 18-24 mo
Enlarging
Positive
Negative
Other imaging modality (CT or MRI)
Biopsy
Arterial hypervasculariarization and venous or delayed-phase washout
Positive
Negative
Return to standard surveillance
Proceed according to lesion size
Treat as hepatocellular carcinoma

Figure 1 Diagnostic algorithm for hepatocellular carcinoma. Modified by Forner *et al*[11]. MDCT: Multidetector computed tomography; MR: Magnetic resonance; CT: Computed tomography; MRI: Magnetic resonance imaging.

60%[15]. A risk of tumor seeding in the path of the puncture has been reported in approximately 2.5% of the cases with a median time of development of 17 mo[16].

HCC staging

The severity of chronic liver disease is usually classified according to the Child-Pugh and model for end-stage liver disease (MELD) scores, and the tumor is staged with the TNM system; in the setting of liver transplantation, the indication for liver replacement is based mainly on the Milan criteria[17]. Another staging system that has gained acceptance is the Barcelona Clinic Liver Cancer (BCLC) score, the advantage of which is that it takes into account the characteristics of the tumor as well as the liver function and the general conditions of the patient[8] (Figure 2). The BCLC system was developed after a retrospective analysis of several cohort studies on patients with HCC at different stages. This system identifies patients with early HCC who may benefit from curative therapies (stage 0 and A), those at intermediate (stage B) or advanced (stage C) stages who may benefit from palliative treatments and those with a very poor life expectancy (stage D). BCLC is the most commonly used staging system in Europe, and it has been approved by the European Association for the Study of the Liver (EASL) and the AASLD.

Multidisciplinary management of HCC

Given the complexity of the clinical scenario, the decision on the most appropriate treatment for a patient with HCC should be made by a multidisciplinary team that includes a hepatologist, hepatobiliary surgeon, transplant surgeon, radiologist and pathologist[18]. No single treatment strategy can be applied to all patients, and treatment should be individualized.

In the management of HCC, attention must be focused on the presence and degree of the underlying chronic liver disease at first observation, which will influence the choice of the treatment[19].

Liver resection can be offered to patients with well-preserved liver function; however, the amount of parenchyma that can be removed in carriers of chronic liver disease is inferior to that which is considered as the safety limit in a normal liver (a “future remnant liver” that is ≥ 30% of the hepatic volume is generally considered acceptable)[19]. Despite a normal liver function, the regenerative potential of a liver that harbors a chronic disease can be surprisingly low.

In cases of impaired liver function, non-surgical procedures or liver transplantation (LT) can be offered; in this setting, the number, size and location of the nodules determine the choice of treatment.

Surgical resection, transplantation and ablation are the treatments that offer the highest rates of complete response and are therefore considered as curative[10]. There are no randomized trials comparing the efficacy of these three approaches, and all evidence is based on the rate of cure reported in different series.

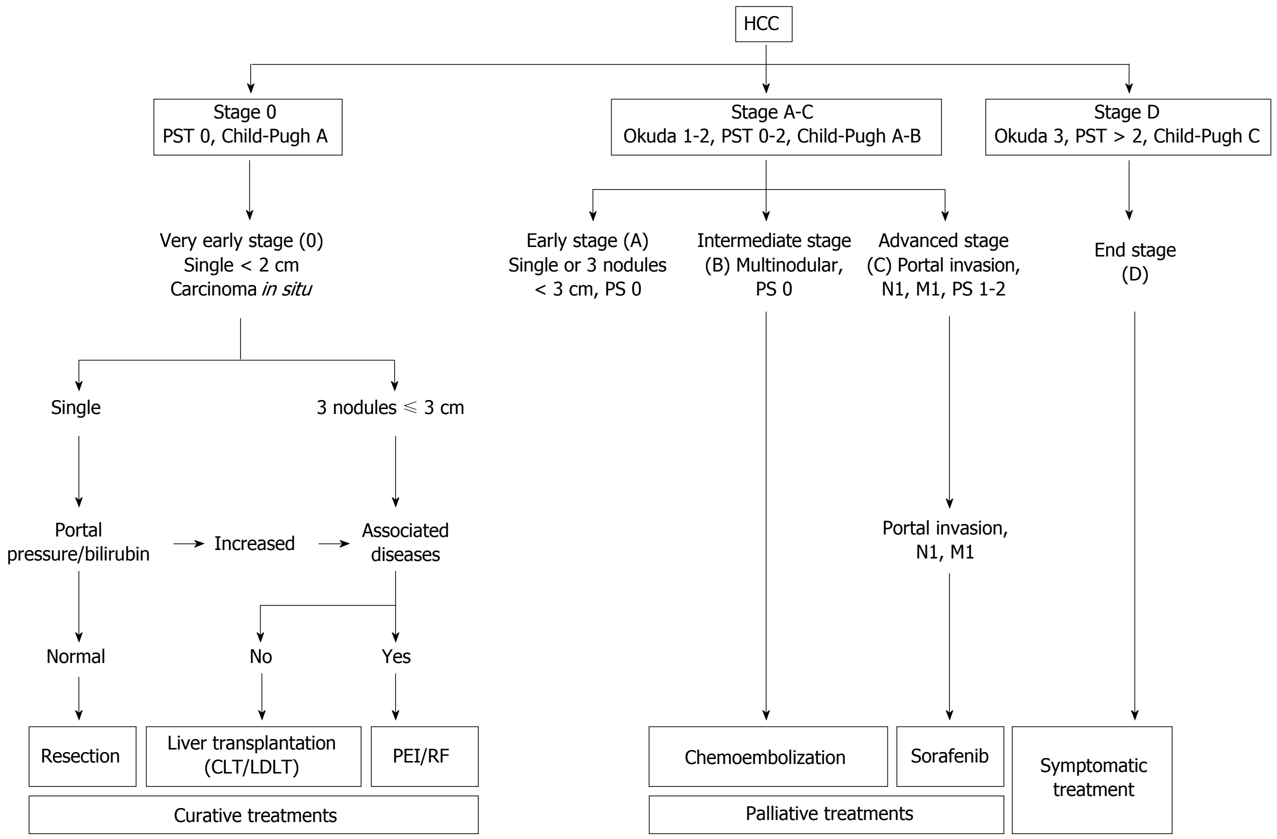

Figure 2 The Barcelona Clinic Liver Cancer staging system and treatment allocation. Copyright © 2010, American Association for the Study of Liver Diseases. CLT: Cadaveric liver transplantation; HCC: Hepatocellular carcinoma; LDLT: Living donor liver transplantation; PEI: Percutaneous ethanol injection; RF: Radiofrequency; PST: Performance status.

Transarterial chemoembolization (TACE) is the least invasive approach; however, it cannot be considered curative.

Ideally, LT is best treatment for early stage HCC on cirrhosis because it removes both the tumor and the chronic disease that has produced it. However, the application of this powerful tool is limited by the scarcity of donors, which results in strict patient selection criteria to optimize the results.

Candidates for LT for whom a long waiting time (> 6 mo) is predicted may be offered resection, local ablation or transarterial chemoembolization as a 'bridge' to transplantation to minimize the risk of tumor progression while they are on the waiting list[20]. The same procedures can also be utilized in attempts downstage tumors that are beyond the eligibility criteria for LT at the time of diagnosis.

Yu *et al*[21] performed a study on HCC patients who exceeded the University of California San Francisco (UCSF) criteria for LT; these patients were downstaged to fit the UCSF criteria using locoregional therapy and finally underwent LT. Patients who were successfully downstaged prior to transplantation had tumor-free and overall survival rates similar to those observed in patients who met the criteria from the beginning.

A 5-year survival comparable to that of "within criteria" HCC patients can be achieved using LT after successful downstaging. Successful downstaging should include tumor size, number of viable tumors and s-αFP concentrations before and after downstaging. Then, a minimum observation period of 3 mo is recommended before considering LT[22].

If a patient's hepatic function allows it, liver resection can be offered prior to future transplantation by pursuing two different strategies: first, resection can be used as the primary therapy with LT offered as a rescue therapy should the patient develop tumor recurrence or postoperative liver failure (salvage transplantation); second, resection can be performed on patients with a high risk of tumor progression while awaiting transplantation (bridge to transplantation)[23,24].

SURGICAL RESECTION

When performed in specialized centers, hepatic resection (HR) can be highly effective, with 5-year overall survival rates well above 50% in the major series[25]. Resection is the recommended treatment for patients without advanced fibrosis as long as an R0 resection can be performed with a low risk of postoperative liver failure[14].

The elements that must be taken into account when considering resection of the cirrhotic liver are the Child-Pugh and MELD scores of the underlying liver disease, the degree of portal hypertension and the extension of the parenchymal excision required to obtain a free resection margin.

The aim of HR is to obtain radical resection with limited surgical morbidity; to achieve this goal, patient selection is crucial. For the last several decades, the selection of candidates for resection has been based on Child-Pugh classification. However, Child-Pugh classification is far from accurate for predicting postoperative liver failure; in fact, some Child-Pugh A patients already have liver functional impairment with an increased bilirubin concentration, clinically significant portal hypertension or even minor fluid retention necessitating diuretic treatment[26]. The further investigation of hepatic functional reserve tests, such as the aminopyrine breath test or clearance of indocyanine green (ICG), has been proposed; however, their predictive value remains poorly validated. In Japan, the ICG retention rate is utilized to identify the best candidates for resection[27], whereas portal pressure and bilirubin are the variables used in Europe and the United States[10]. Recently, a preoperative MELD score $\geqslant$ 10 was associated with a higher incidence (40%) of postoperative liver failure[28].

Markers of portal hypertension including a porto-caval gradient > 10 mmHg, the presence of esophageal varices, splenomegaly and a platelet count lower than 1×10^{11}/L are predictors of postoperative morbidity and mortality[29]. In patients without relevant portal hypertension and normal concentrations of bilirubin, the 5-year survival is 70%, whereas this value is 50% for individuals with portal hypertension and is even lower when both these risk factors are present[30,31].

By integrating all of these factors, HR can be safely performed on patients with Child-Pugh class A chronic liver disease, a MELD score $\leqslant$ 10, a platelet count > 100.000/mm^3 and a porto-caval gradient <10 mmHg. These factors dramatically limit the potential number of candidates, and overall, less than 30% of patients are candidates for HR[29].

After HR, the 5-year survival for cirrhotic patients with HCC ranges from 30% to 50%, whereas the operative mortality ranges from 3% to 8%[29]. The severity of cirrhosis, size of the tumor, number of tumors, presence of vascular tumor invasion and presence of satellite nodules are well-established prognostic factors for recurrence and survival[27,32,33]. Late recurrence is mainly due to the carcinogenic effect of underlying chronic liver disease[34].

Absolute contraindications to HR are the presence of extrahepatic metastases or neoplastic invasion of the main portal trunk. Neoplastic portal vein thrombosis is a poor prognostic factor; however, in highly selected cases, hemi-hepatectomy can be feasible, particularly when thrombosis of a main branch of the portal vein has led to hypertrophy of the contralateral hemiliver.

When compared with open surgery, laparoscopy in cirrhotic patients could have the advantage of avoiding the interruption of collateral abdominal veins that are present as a result of portal hypertension. Several studies have indeed demonstrated the benefits of the laparoscopic approach in terms reduced bleeding and lower postoperative morbidity and mortality[35,36].

LIVER TRANSPLANTATION

HCC is the only solid cancer that can be treated with transplantation. Transplantation is the best curative option for patients with decompensated (Child-Pugh B or C) cirrhosis; however, due to the shortage of donors, it can only be offered to a limited number of patients. Candidates for LT are patients with tumors that have favorable pathological features and therefore a low likelihood of recurrence.

The most widely adopted criteria for selecting HCC carriers for transplantation are the Milan criteria. According to these criteria, LT can be considered only for patients with a single tumor < 5 cm in diameter or for patients with up to 3 tumors < 3 cm without macrovascular invasion[17]. A recent systematic review of 90 studies that followed 17780 patients over a 15-year period identified the Milan criteria as an independent prognostic factor of outcome after LT[37].

Despite recent discussions concerning the restrictive nature of the Milan criteria, these criteria have been adopted by the vast majority of transplant centers. The results of LT in HCC within the Milan criteria are outstanding, with 5-year survival approaching 80%, which is similar to that observed in patients transplanted for benign diseases[30,38,39]. Outside the Milan criteria, survival is significantly reduced, which is likely due to an increased prevalence of variables associated with risk of recurrence, such as microvascular invasion, in tumors at more advanced stages[40].

Neoadjuvant therapy through TACE can occasionally downstage tumors that were outside the Milan criteria at the time of diagnosis; in these cases, the results of LT are similar to those achieved when the criteria were met at first observation[41].

The time spent on the waiting list is a key factor that must be considered when assessing the results of LT in HCC patients; it depends on the availability of donors in a given area and on the system used to prioritize organ allocation. It was demonstrated that 15%-20% of patients with HCC initially within the Milan criteria experience tumor progression until they dropped out from the waiting list; this highlights the need to analyze the results of LT using an intention to treat approach[42]. Although treatments aimed at delaying tumor progression, such as ablation and transarterial chemoembolization, are widely used, their efficacy is unproven[43]. Live donation is a valid strategy for extending the donor pool; however, its applicability is reduced because of societal constraints, scarcity of appropriate donors and the possible morbidity and mortality of donors[44].

Currently, organs are allocated worldwide based on MELD score; however, HCC patients with low MELD scores are given extra points to shorten their waiting time and avoid tumor progression. This policy is questioned by some authors because it might be disadvantageous for patients without HCC[45].

LOCOREGIONAL TREATMENTS

Local ablation techniques have been developed for patients with surgical contraindications and can be performed either through a percutaneous approach or, less commonly, through laparoscopy. These maneuvers include percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), microwave ablation, cryoablation, laser-induced thermotherapy, high-intensity focused ultrasound and irreversible electroporation[46].

The first percutaneous treatment was PEI, which induces coagulative necrosis of the lesion as a result of cellular dehydration, protein denaturation and chemical occlusion of small tumor vessels due to the effects of the injected absolute alcohol. Ablation techniques such as RFA, microwave ablation, and laser ablation utilize high temperatures; conversely, cryoablation causes direct tumor freezing[47].

The evaluation of responses to locoregional treatments and molecular-targeted therapies of HCC is currently based on modified RECIST (mRECIST) criteria, which measure the diameter of the viable tumor component of target lesions[48].

Radiofrequency ablation

RFA is the technique of choice for local destruction of liver tumors. RFA induces coagulative necrosis of the tumor with safety margins around the lesion and is the most commonly used local ablative technique. RFA has largely replaced PEI because it produces better results in terms of recurrence-free survival and requires fewer treatment sessions[49]. RFA can be performed percutaneously under imaging guidance (ultrasound, CT or MRI) or during surgery guided by intraoperative US. The advantage of RF in the treatment of HCC in cirrhotic patients is that it allows selective destruction of the tumor, sparing the surrounding parenchyma, and can be easily repeated in case of recurrence. Complete ablation of lesions smaller than 2 cm is possible in more than 90% of cases[50].

There are several major limits to the use of RF: (1) complete necrosis is rarely observed when the tumor diameter is > 3 cm or when the tumor is adjacent to a major blood vessel due to the cooling effect of the blood flow; (2) it is difficult to reach some areas of the liver parenchyma percutaneously (*e.g.*, segment 1); (3) subcapsular lesions can undergo rupture in the peritoneum; (4) bladder injury can occur when lesions in segments IVb-5 are treated; and (5) targeting the lesion can be difficult under ultrasound guidance in livers with multinodular cirrhosis.

Taking these limitations into account, the benefit of RF in the treatment of HCC has been well demonstrated, with overall 5-year survival rates between 33% and 55% in selected series[51]. The effectiveness of RFA has led to the proposal of this technique as an alternative to HR.

In the only randomized prospective trial with balanced groups of patients comparing HR to RFA for HCC < 3 cm in patients with cirrhosis, no difference was observed in terms of overall and disease-free survival, whereas RF was associated with lower perioperative morbidity (4.2% *vs* 55.5%, $P < 0.05$) and mortality (0% *vs* 1.1%)[52]. However, in a retrospective comparative study of Child-Pugh class A patients, surgery was significantly more effective for patients with single tumors > 3 cm in diameter, with an overall 3-year survival of 66% after surgery (*vs* 37% after RFA, $P = 0.004$) and a 3-year disease-free survival of 44% (*vs* 19%, $P = 0.001$)[53]. This observation was confirmed by subsequent studies performed by different groups[54-56]. A recent systematic review and meta-analysis of 12 controlled trials showed a not notable difference in the short-term effectiveness of RFA and HR in the treatment of early-stage hepatocellular carcinoma meeting Milan criteria, but the long-term efficacy of HR was better than that of RFA[57]. However, HR was associated with more complications and a longer hospital stay.

Rather than competing techniques, RFA and HR are effective therapeutic options that can be chosen based on the severity of chronic liver disease as well as the size and location of the tumor.

Transarterial chemoembolization

Transarterial chemoembolization (TACE) is the most commonly used initial treatment for unresectable HCC[58] and is also the first-line therapy for downstaging tumors that exceed the criteria for transplantation or to avoid tumor progression in patients awaiting LT. TACE may also be considered as a neoadjuvant treatment that can be utilized before HR or RF ablation to reduce tumor volume and possibly target satellite micrometastases[59].

The rationale behind TACE use is the well-characterized angiogenic activity of HCC that results in hypervascular arterial feeding. This technique depends on the intra-arterial infusion of a cytotoxic chemotherapeutic agent emulsioned with Lipiodol followed by embolization of the feeding vessels through a trans-arterial catheter[60]. TACE is a well-established treatment for HCC in cirrhotic patients, and its efficacy for improving survival compared with the other supporting treatments has been demonstrated[61].

The maximum and sustained retention of the chemotherapeutic agent is used as a measure of the success of TACE; thus, embolic microspheres are employed that have the ability to sequester chemotherapeutic agents. The concentration of these microspheres is increased within the tumor, and their contents are subsequently released in a controlled manner over a 1-wk period, which reduces the systemic toxicity to a minimum[62].

Doxorubicin-eluting beads (DEB) are another trans-arterial liver-directed therapy. The use of an eluting bead can be considered an improvement over conventional TACE. DEB are preformed, deformable microspheres that are loaded with doxorubicin (up to 150 mg per treatment). The pharmacokinetic profile of DEB significantly differs from that of conventional TACE; in particular, the peak drug concentration in the serum is lower for DEB-

TACE compared with conventional TACE. An objective response rate of 70% to 80% according to the EASL criteria has been achieved[63]. One- and 3-year survival rates of 89.9% and 66.3%, respectively, have been reported in a heterogeneous cohort of patients with BCLC (stages A to C) treated with DEB-TACE[64].

Absolute contraindications for TACE are decompensated cirrhosis (Child-Pugh B $\geqslant$ 8, including jaundice, clinical encephalopathy and refractory ascites), extensive tumor with massive replacement of both lobes in their entirety, severely reduced portal vein flow (portal vein occlusion or hepatofugal blood flow), and a creatinine clearance < 30 mL/min[65].

Microwave ablation

Microwave ablation (MWA) is a potentially curative ablation procedure that has been proven to be safe in both percutaneous and intraoperative settings[66]. MWA can be utilized in patients with advanced liver disease and HCC, provides a more predictable ablation compared with RFA and requires fewer treatment sessions[21,67,68].

Microwave ablation creates an electromagnetic field in the tissues surrounding the ablation antenna with an extension of several centimeter, without flow of electrical current. Tissue within the MWA field heats rapidly to temperatures over 100 ℃ without the detrimental effects of tissue impedance, allowing a more rapid and consistent ablation[10]. Microwave ablation carries the risk of more severe injury to adjacent structures due to differences in energy delivery when compared with RFA; therefore, the operative approach is preferred over the percutaneous approach because it allows liver mobilization and protection of adjacent organs[66,69,70].

Radioembolization

Radioembolization is a newer hepatic transarterial technique that employs radioactive substances such as Iodine-131-labeled Lipiodol[71] or microspheres containing Yttrium-90[72]. This technique has been shown to be feasible and safe for the treatment of HCC in cirrhotic patients[73,74]. Microspheres are delivered to the tumor area for selective production of high energy and low penetration radiation. Radioembolization can be safely performed in patients with portal vein thrombosis due to the minimally embolic effect of 90Y microspheres[75]. The reported rate of complete tumor necrosis is 90% for patients with HCC < 3 cm[76], whereas the rate of complete necrosis after TACE varies widely in the literature, from 15% to 70%[77].

Radiation therapy

Radiation therapy is generally not considered an option in HCC consensus documents or national guidelines, primarily because of the lack of level 1 evidence[78]. However, experience with conformal radiation therapy (RT), intensity modulated RT, stereotactic body RT and particle therapy is rapidly increasing. RT should be considered as a treatment option in patients unsuitable for other established local therapies[78]. RT has also been used as a bridge to liver transplant and can be safely combined with locoregional therapies such as TACE[78,79].

SYSTEMIC TREATMENTS

Systemic treatment of HCC in cirrhotic patients is not effective due to the poor chemosensitivity of the tumor, and the impairment of hepatic function significantly increases the toxicity of chemotherapy.

Because the demonstrated benefits of systemic chemotherapy and hormonal treatments are lacking[80,81], molecular targeted therapies have recently been developed. Sorafenib, an inhibitor of multi-kinase, has antiproliferative and antiangiogenic activity, delays tumor progression and is currently the only agent with proven efficacy for the treatment of patients with advanced HCC[11,82-84]. This multitargeted tyrosine kinase inhibitor is a small molecule that inhibits vascular endothelial growth factor receptor, platelet-derived growth factor receptor, B-Raf, Fms-related tyrosine kinase and c-kit[85,86].

The use of sorafenib is currently recommended for patients with preserved liver function and advanced HCC who are not suitable for HR or LT and have failed to respond to locoregional treatments[87]. It is recommended that the treatment be continued until progression of the tumor is demonstrated. The main side effects associated with the use of sorafenib are diarrhea and hand-foot skin reaction; other possible side effects include anorexia, nausea, vomiting, weight loss, hoarseness of voice, asthenia and hypertension[52,88]. These effects can occasionally require dose reduction or treatment discontinuation. The potential benefit of sorafenib as adjuvant treatment after LT has not been demonstrated.

On the basis of a large randomized phase Ⅲ study, the Sorafenib HCC Assessment Randomized Protocol[83], Sorafenib has been approved by the United States Food and Drug Administration for the treatment of patients with advanced HCC.

Studies are ongoing that aim to identify the best responders to Sorafenib; c-Jun N-terminal kinase (JNK) activity was positively correlated with the CD133 expression level and inversely correlated with the therapeutic response to Sorafenib. Accordingly, JNK activity may be considered as a new predictive biomarker for response to Sorafenib treatment[89].

To date, there is no second-line treatment for patients who are intolerant to Sorafenib or experience tumor progression while undergoing treatment.

COMBINED TREATMENTS

A combination of the different therapeutic approaches mentioned thus far is often required to address the different clinical peculiarities of HCC patients. For example, chemoembolization or RFA can be performed before radical surgery with curative intent (HR or LT) to allow effective tumor downstaging or reduce tumor growth. Different authors have proposed HR as a first-line therapy in patients who are candidates for LT[90]; however, the possible effect of previous surgery on the technical complexity of the liver transplantation procedure is a matter of debate[90,91]. One argument in favor of hepatic resection prior to LT is that histological analysis of the tumor can provide useful information regarding its oncological behavior: features such as the presence of a tumor capsule, the degree of differentiation or the presence of micro-vascular invasion can be precisely assessed in the surgical specimen and are well-known prognostic factors. However, there is no consensus among different authors regarding the influence that histological parameters should have on the therapeutic strategy; some authors suggest that LT should be contraindicated for patients with tumors that display poor prognostic histological criteria, whereas others recommend transplant priority in the presence of the same risk factors[23,92].

CONCLUSION

The treatment of HCC in cirrhotic patients has changed significantly over the past several decades and has become a major clinical issue. Patients with HCC on cirrhosis can benefit from several effective treatments that will improve their survival; however, the choice of the most appropriate options depends on several factors, namely: (1) severity of the underlying chronic liver disease; (2) stage of the tumor assessed *via* imaging; (3) histological features of the tumor (when available); (4) availability of an active transplant program; (5) availability of a hepatobiliary surgical unit; and (6) availability of an experienced interventional radiology service.

Some of these variables may account for the different attitudes that are often observed. To ensure that the most effective treatment can be offered for a given case, a multidisciplinary approach is warranted, and professionals skilled in the administration of the different treatment types should be available. The increase in the incidence of HCC justifies the development of services related to the management of this tumor.

REFERENCES

1 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]

2 **Yang HI**, Sherman M, Su J, Chen PJ, Liaw YF, Iloeje UH, Chen CJ. Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *J Clin Oncol* 2010; **28**: 2437-2444 [PMID: 20368541 DOI: 10.1200/JCO.2009.27.4456]

3 **Lok AS**, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138-148 [PMID: 18848939 DOI: 10.1053/j.gastro.2008.09.014]

4 **Bouchard MJ**, Navas-Martin S. Hepatitis B and C virus hepatocarcinogenesis: lessons learned and future challenges. *Cancer Lett* 2011; **305**: 123-143 [PMID: 21168955 DOI: 10.1016/j.canlet.2010.11.014]

5 **Bruno S**, Stroffolini T, Colombo M, Bollani S, Benvegnù L, Mazzella G, Ascione A, Santantonio T, Piccinino F, Andreone P, Mangia A, Gaeta GB, Persico M, Fagiuoli S, Almasio PL. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; **45**: 579-587 [PMID: 17326216 DOI: 10.1002/hep.21492]

6 **Singal A**, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009; **30**: 37-47 [PMID: 19392863 DOI: 10.1111/j.1365-2036.2009.04014.x]

7 **Bolondi L**. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076-1084 [PMID: 14642630]

8 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

9 **Zhang BH**, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; **130**: 417-422 [PMID: 15042359 DOI: 10.1007/s00432-004-0552-0]

10 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

11 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]

12 **Sangiovanni A**, Manini MA, Iavarone M, Romeo R, Forzenigo LV, Fraquelli M, Massironi S, Della Corte C, Ronchi G, Rumi MG, Biondetti P, Colombo M. The diagnostic and economic impact of contrast imaging techniques in the diagnosis of small hepatocellular carcinoma in cirrhosis. *Gut* 2010; **59**: 638-644 [PMID: 19951909 DOI: 10.1136/gut.2009.187286]

13 **Davis GL**, Dempster J, Meler JD, Orr DW, Walberg MW, Brown B, Berger BD, O'Connor JK, Goldstein RM. Hepatocellular carcinoma: management of an increasingly common problem. *Proc (Bayl Univ Med Cent)* 2008; **21**: 266-280 [PMID: 18628926]

14 **Verslype C**, Rosmorduc O, Rougier P. Hepatocellular carcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23** Suppl 7: vii42-vii48 [PMID: 22997453 DOI: 10.1093/annonc/mds225]

15 **Silva MA**, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* 2008; **57**: 1592-1596 [PMID: 18669577 DOI: 10.1136/gut.2008.149062]

16 **Durand F**, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, Moutardier V, Farges O, Valla D. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. *J Hepatol* 2001; **35**: 254-258 [PMID: 11580148]

17 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

18 **Gish RG**, Lencioni R, Di Bisceglie AM, Raoul JL, Mazzaferro V. Role of the multidisciplinary team in the diagnosis and treatment of hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* 2012; **6**: 173-185 [PMID: 22375523 DOI: 10.1586/egh.11.105]

19 **Vauthey JN**, Dixon E, Abdalla EK, Helton WS, Pawlik TM, Taouli B, Brouquet A, Adams RB. Pretreatment assessment of hepatocellular carcinoma: expert consensus statement. *HPB (Oxford)* 2010; **12**: 289-299 [PMID: 20590901 DOI: 10.1111/j.1477-2574.2010.00181.x]

20 **Belghiti J**, Carr BI, Greig PD, Lencioni R, Poon RT. Treatment before liver transplantation for HCC. *Ann Surg*

Oncol 2008; **15**: 993-1000 [PMID: 18236111 DOI: 10.1245/s10434-007-9787-8]

21 **Yu CY**, Ou HY, Huang TL, Chen TY, Tsang LL, Chen CL, Cheng YF. Hepatocellular carcinoma downstaging in liver transplantation. *Transplant Proc* 2012; **44**: 412-414 [PMID: 22410030 DOI: 10.1016/j.transproceed.2012.01.043]

22 **Ravaioli M**, Cucchetti A, Cescon M, Piscaglia F, Ercolani G, Trevisani F, Pinna AD. Systematic review of outcome of downstaging hepatocellular cancer before liver transplantation in patients outside the Milan criteria (Br J Surg 2011; 98: 1201-1208). *Br J Surg* 2011; **98**: 1674; author reply 1675 [PMID: 21964691 DOI: 10.1002/bjs.7728]

23 **Sala M**, Fuster J, Llovet JM, Navasa M, Solé M, Varela M, Pons F, Rimola A, García-Valdecasas JC, Brú C, Bruix J. High pathological risk of recurrence after surgical resection for hepatocellular carcinoma: an indication for salvage liver transplantation. *Liver Transpl* 2004; **10**: 1294-1300 [PMID: 15376311 DOI: 10.1002/lt.20202]

24 **Cucchetti A**, Vitale A, Gaudio MD, Ravaioli M, Ercolani G, Cescon M, Zanello M, Morelli MC, Cillo U, Grazi GL, Pinna AD. Harm and benefits of primary liver resection and salvage transplantation for hepatocellular carcinoma. *Am J Transplant* 2010; **10**: 619-627 [PMID: 20121741 DOI: 10.1111/j.1600-6143.2009.02984.x]

25 **Lim KC**, Chow PK, Allen JC, Siddiqui FJ, Chan ES, Tan SB. Systematic review of outcomes of liver resection for early hepatocellular carcinoma within the Milan criteria. *Br J Surg* 2012; **99**: 1622-1629 [PMID: 23023956 DOI: 10.1002/bjs.8915]

26 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]

27 **Llovet JM**, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 2005; **25**: 181-200 [PMID: 15918147 DOI: 10.1055/s-2005-871198]

28 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, Ramacciato G, Grazi GL, Pinna AD. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009; **250**: 922-928 [PMID: 19855258 DOI: 10.1097/SLA.0b013e3181b977a5]

29 **Belghiti J**, Kianmanesh R. Surgical treatment of hepatocellular carcinoma. *HPB (Oxford)* 2005; **7**: 42-49 [PMID: 18333160 DOI: 10.1080/13651820410024067]

30 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522 DOI: 10.1002/hep.510300629]

31 **Ishizawa T**, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, Imamura H, Sugawara Y, Kokudo N, Makuuchi M. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1908-1916 [PMID: 18549877 DOI: 10.1053/j.gastro.2008.02.091]

32 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: 12547409]

33 **Fancellu A**, Rosman AS, Sanna V, Nigri GR, Zorcolo L, Pisano M, Melis M. Meta-analysis of trials comparing minimally-invasive and open liver resections for hepatocellular carcinoma. *J Surg Res* 2011; **171**: e33-e45 [PMID: 21920552 DOI: 10.1016/j.jss.2011.07.008]

34 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]

35 **Rao A**, Rao G, Ahmed I. Laparoscopic or open liver resection? Let systematic review decide it. *Am J Surg* 2012; **204**: 222-231 [PMID: 22245507 DOI: 10.1016/j.amjsurg.2011.08.013]

36 **Kluger MD**, Cherqui D. Laparoscopic resection of hepatocellular carcinoma. *Recent Results Cancer Res* 2013; **190**: 111-126 [PMID: 22941017 DOI: 10.1007/978-3-642-16037-0_8]

37 **Mazzaferro V**, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]

38 **Bismuth H**, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Ann Surg* 1993; **218**: 145-151 [PMID: 8393649]

39 **Jonas S**, Bechstein WO, Steinmüller T, Herrmann M, Radke C, Berg T, Settmacher U, Neuhaus P. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology* 2001; **33**: 1080-1086 [PMID: 11343235 DOI: 10.1053/jhep.2001.23561]

40 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]

41 **Majno PE**, Adam R, Bismuth H, Castaing D, Ariche A, Krissat J, Perrin H, Azoulay D. Influence of preoperative transarterial lipiodol chemoembolization on resection and transplantation for hepatocellular carcinoma in patients with cirrhosis. *Ann Surg* 1997; **226**: 688-701; discussion 701-3 [PMID: 9409568]

42 **Llovet JM**, Bruix J, Gores GJ. Surgical resection versus transplantation for early hepatocellular carcinoma: clues for the best strategy. *Hepatology* 2000; **31**: 1019-1021 [PMID: 10733561 DOI: 10.1053/he.2000.6959]

43 **Llovet JM**, Mas X, Aponte JJ, Fuster J, Navasa M, Christensen E, Rodés J, Bruix J. Cost effectiveness of adjuvant therapy for hepatocellular carcinoma during the waiting list for liver transplantation. *Gut* 2002; **50**: 123-128 [PMID: 11772979]

44 **Middleton PF**, Duffield M, Lynch SV, Padbury RT, House T, Stanton P, Verran D, Maddern G. Living donor liver transplantation--adult donor outcomes: a systematic review. *Liver Transpl* 2006; **12**: 24-30 [PMID: 16498709]

45 **Goldberg D**, French B, Abt P, Feng S, Cameron AM. Increasing disparity in waitlist mortality rates with increased model for end-stage liver disease scores for candidates with hepatocellular carcinoma versus candidates without hepatocellular carcinoma. *Liver Transpl* 2012; **18**: 434-443 [PMID: 22271656 DOI: 10.1002/lt.23394]

46 **Padma S**, Martinie JB, Iannitti DA. Liver tumor ablation: percutaneous and open approaches. *J Surg Oncol* 2009; **100**: 619-634 [PMID: 20017157 DOI: 10.1002/jso.21364]

47 **Lencioni R**. Loco-regional treatment of hepatocellular carcinoma. *Hepatology* 2010; **52**: 762-773 [PMID: 20564355 DOI: 10.1002/hep.23725]

48 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]

49 **Brunello F**, Veltri A, Carucci P, Pagano E, Ciccone G, Moretto P, Sacchetto P, Gandini G, Rizzetto M. Radiofrequency ablation versus ethanol injection for early hepatocellular car-

cinoma: A randomized controlled trial. *Scand J Gastroenterol* 2008; **43**: 727-735 [PMID: 18569991 DOI: 10.1080/00365520701885481]

50 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]

51 **Lencioni R**, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005; **234**: 961-967 [PMID: 15665226 DOI: 10.1148/radiol.2343040350]

52 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328 [PMID: 16495695 DOI: 10.1097/01.sla.0000201480.65519.b8]

53 **Vivarelli M**, Guglielmi A, Ruzzenente A, Cucchetti A, Bellusci R, Cordiano C, Cavallari A. Surgical resection versus percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma on cirrhotic liver. *Ann Surg* 2004; **240**: 102-107 [PMID: 15213625]

54 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100 DOI: 10.1097/SLA.0b013e3181efc656]

55 **Imai K**, Beppu T, Chikamoto A, Doi K, Okabe H, Hayashi H, Nitta H, Ishiko T, Takamori H, Baba H. Comparison between hepatic resection and radiofrequency ablation as first-line treatment for solitary small-sized hepatocellular carcinoma of 3 cm or less. *Hepatol Res* 2013; **43**: 853-864 [PMID: 23281579 DOI: 10.1111/hepr.12035]

56 **Parisi A**, Desiderio J, Trastulli S, Castellani E, Pasquale R, Cirocchi R, Boselli C, Noya G. Liver resection versus radiofrequency ablation in the treatment of cirrhotic patients with hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2013; **12**: 270-277 [PMID: 23742772]

57 **Duan C**, Liu M, Zhang Z, Ma K, Bie P. Radiofrequency ablation versus hepatic resection for the treatment of early-stage hepatocellular carcinoma meeting Milan criteria: a systematic review and meta-analysis. *World J Surg Oncol* 2013; **11**: 190 [PMID: 23941614 DOI: 10.1186/1477-7819-11-190]

58 **Takayasu K**, Arii S, Ikai I, Omata M, Okita K, Ichida T, Matsuyama Y, Nakanuma Y, Kojiro M, Makuuchi M, Yamaoka Y. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; **131**: 461-469 [PMID: 16890600 DOI: 10.1053/j.gastro.2006.05.021]

59 **Schwartz M**, Roayaie S, Konstadoulakis M. Strategies for the management of hepatocellular carcinoma. *Nat Clin Pract Oncol* 2007; **4**: 424-432 [PMID: 17597707 DOI: 10.1038/ncponc0844]

60 **Goldberg SN**, Grassi CJ, Cardella JF, Charboneau JW, Dodd GD, Dupuy DE, Gervais DA, Gillams AR, Kane RA, Lee FT, Livraghi T, McGahan J, Phillips DA, Rhim H, Silverman SG, Solbiati L, Vogl TJ, Wood BJ, Vedantham S, Sacks D. Image-guided tumor ablation: standardization of terminology and reporting criteria. *J Vasc Interv Radiol* 2009; **20**: S377-S390 [PMID: 19560026 DOI: 10.1016/j.jvir.2009.04.011]

61 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]

62 **Varela M**, Real MI, Burrel M, Forner A, Sala M, Brunet M, Ayuso C, Castells L, Montañá X, Llovet JM, Bruix J. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; **46**: 474-481 [PMID: 17239480 DOI: 10.1016/j.jhep.2006.10.020]

63 **Forner A**, Ayuso C, Varela M, Rimola J, Hessheimer AJ, de Lope CR, Reig M, Bianchi L, Llovet JM, Bruix J. Evaluation of tumor response after locoregional therapies in hepatocellular carcinoma: are response evaluation criteria in solid tumors reliable? *Cancer* 2009; **115**: 616-623 [PMID: 19117042 DOI: 10.1002/cncr.24050]

64 **Burrel M**, Reig M, Forner A, Barrufet M, de Lope CR, Tremosini S, Ayuso C, Llovet JM, Real MI, Bruix J. Survival of patients with hepatocellular carcinoma treated by transarterial chemoembolisation (TACE) using Drug Eluting Beads. Implications for clinical practice and trial design. *J Hepatol* 2012; **56**: 1330-1335 [PMID: 22314428 DOI: 10.1016/j.jhep.2012.01.008]

65 **Raoul JL**, Sangro B, Forner A, Mazzaferro V, Piscaglia F, Bolondi L, Lencioni R. Evolving strategies for the management of intermediate-stage hepatocellular carcinoma: available evidence and expert opinion on the use of transarterial chemoembolization. *Cancer Treat Rev* 2011; **37**: 212-220 [PMID: 20724077 DOI: 10.1016/j.ctrv.2010.07.006]

66 **Bertot LC**, Sato M, Tateishi R, Yoshida H, Koike K. Mortality and complication rates of percutaneous ablative techniques for the treatment of liver tumors: a systematic review. *Eur Radiol* 2011; **21**: 2584-2596 [PMID: 21858539 DOI: 10.1007/s00330-011-2222-3]

67 **Wright AS**, Sampson LA, Warner TF, Mahvi DM, Lee FT. Radiofrequency versus microwave ablation in a hepatic porcine model. *Radiology* 2005; **236**: 132-139 [PMID: 15987969 DOI: 10.1148/radiol.2361031249]

68 **Simon CJ**, Dupuy DE, Mayo-Smith WW. Microwave ablation: principles and applications. *Radiographics* 2005; **25** Suppl 1: S69-S83 [PMID: 16227498 DOI: 10.1148/rg.25si055501]

69 **Liang P**, Wang Y, Yu X, Dong B. Malignant liver tumors: treatment with percutaneous microwave ablation--complications among cohort of 1136 patients. *Radiology* 2009; **251**: 933-940 [PMID: 19304921 DOI: 10.1148/radiol.2513081740]

70 **Swan RZ**, Sindram D, Martinie JB, Iannitti DA. Operative microwave ablation for hepatocellular carcinoma: complications, recurrence, and long-term outcomes. *J Gastrointest Surg* 2013; **17**: 719-729 [PMID: 23404173 DOI: 10.1007/s11605-013-2164-y]

71 **Raoul JL**, Guyader D, Bretagne JF, Heautot JF, Duvauferrier R, Bourguet P, Bekhechi D, Deugnier YM, Gosselin M. Prospective randomized trial of chemoembolization versus intra-arterial injection of 131I-labeled-iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997; **26**: 1156-1161 [PMID: 9362356 DOI: 10.1002/hep.510260511]

72 **Salem R**, Lewandowski RJ, Mulcahy MF, Riaz A, Ryu RK, Ibrahim S, Atassi B, Baker T, Gates V, Miller FH, Sato KT, Wang E, Gupta R, Benson AB, Newman SB, Omary RA, Abecassis M, Kulik L. Radioembolization for hepatocellular carcinoma using Yttrium-90 microspheres: a comprehensive report of long-term outcomes. *Gastroenterology* 2010; **138**: 52-64 [PMID: 19766639 DOI: 10.1053/j.gastro.2009.09.006]

73 **Sangro B**, Carpanese L, Cianni R, Golfieri R, Gasparini D, Ezziddin S, Paprottka PM, Fiore F, Van Buskirk M, Bilbao JI, Ettorre GM, Salvatori R, Giampalma E, Geatti O, Wilhelm K, Hoffmann RT, Izzo F, Iñarrairaegui M, Maini CL, Urigo C, Cappelli A, Vit A, Ahmadzadehfar H, Jakobs TF, Lastoria S. Survival after yttrium-90 resin microsphere radioembolization of hepatocellular carcinoma across Barcelona clinic liver cancer stages: a European evaluation. *Hepatology* 2011; **54**: 868-878 [PMID: 21618574 DOI: 10.1002/hep.24451]

74 **Vente MA**, Wondergem M, van der Tweel I, van den Bosch MA, Zonnenberg BA, Lam MG, van Het Schip AD, Nijsen

JF. Yttrium-90 microsphere radioembolization for the treatment of liver malignancies: a structured meta-analysis. *Eur Radiol* 2009; **19**: 951-959 [PMID: 18989675 DOI: 10.1007/s00330-008-1211-7]

75 **Kulik LM**, Carr BI, Mulcahy MF, Lewandowski RJ, Atassi B, Ryu RK, Sato KT, Benson A, Nemcek AA, Gates VL, Abecassis M, Omary RA, Salem R. Safety and efficacy of 90Y radiotherapy for hepatocellular carcinoma with and without portal vein thrombosis. *Hepatology* 2008; **47**: 71-81 [PMID: 18027884 DOI: 10.1002/hep.21980]

76 **Riaz A**, Kulik L, Lewandowski RJ, Ryu RK, Giakoumis Spear G, Mulcahy MF, Abecassis M, Baker T, Gates V, Nayar R, Miller FH, Sato KT, Omary RA, Salem R. Radiologic-pathologic correlation of hepatocellular carcinoma treated with internal radiation using yttrium-90 microspheres. *Hepatology* 2009; **49**: 1185-1193 [PMID: 19133645 DOI: 10.1002/hep.22747]

77 **Gerunda GE**, Neri D, Merenda R, Barbazza F, Zangrandi F, Meduri F, Bisello M, Valmasoni M, Gangemi A, Faccioli AM. Role of transarterial chemoembolization before liver resection for hepatocarcinoma. *Liver Transpl* 2000; **6**: 619-626 [PMID: 10980062 DOI: 10.1053/jlts.2000.8312]

78 **Klein J**, Dawson LA. Hepatocellular carcinoma radiation therapy: review of evidence and future opportunities. *Int J Radiat Oncol Biol Phys* 2013; **87**: 22-32 [PMID: 23219567 DOI: 10.1016/j.ijrobp.2012.08.043]

79 **Koo JE**, Kim JH, Lim YS, Park SJ, Won HJ, Sung KB, Suh DJ. Combination of transarterial chemoembolization and three-dimensional conformal radiotherapy for hepatocellular carcinoma with inferior vena cava tumor thrombus. *Int J Radiat Oncol Biol Phys* 2010; **78**: 180-187 [PMID: 19926229 DOI: 10.1016/j.ijrobp.2009.07.1730]

80 **Taieb J**, Barbare JC, Rougier P. Medical treatments for hepatocellular carcinoma (HCC): what's next? *Ann Oncol* 2006; **17** Suppl 10: x308-x314 [PMID: 17018744 DOI: 10.1093/annonc/mdl279]

81 **Chow PK**, Tai BC, Tan CK, Machin D, Win KM, Johnson PJ, Soo KC. High-dose tamoxifen in the treatment of inoperable hepatocellular carcinoma: A multicenter randomized controlled trial. *Hepatology* 2002; **36**: 1221-1226 [PMID: 12395333 DOI: 10.1053/jhep.2002.36824]

82 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

83 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

84 **Lencioni R**, Kudo M, Ye SL, Bronowicki JP, Chen XP, Dagher L, Furuse J, Geschwind JF, Ladrón de Guevara L, Papandreou C, Sanyal AJ, Takayama T, Yoon SK, Nakajima K, Cihon F, Heldner S, Marrero JA. First interim analysis of the GIDEON (Global Investigation of therapeutic decisions in hepatocellular carcinoma and of its treatment with sorafeNib) non-interventional study. *Int J Clin Pract* 2012; **66**: 675-683 [PMID: 22698419 DOI: 10.1111/j.1742-1241.2012.02940.x]

85 **Chang YS**, Adnane J, Trail PA, Levy J, Henderson A, Xue D, Bortolon E, Ichetovkin M, Chen C, McNabola A, Wilkie D, Carter CA, Taylor IC, Lynch M, Wilhelm S. Sorafenib (BAY 43-9006) inhibits tumor growth and vascularization and induces tumor apoptosis and hypoxia in RCC xenograft models. *Cancer Chemother Pharmacol* 2007; **59**: 561-574 [PMID: 17160391 DOI: 10.1007/s00280-006-0393-4]

86 **Wilhelm SM**, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004; **64**: 7099-7109 [PMID: 15466206 DOI: 10.1158/0008-5472.CAN-04-1443]

87 **European Association For The Study Of The Liver**, European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

88 **Xie B**, Wang DH, Spechler SJ. Sorafenib for treatment of hepatocellular carcinoma: a systematic review. *Dig Dis Sci* 2012; **57**: 1122-1129 [PMID: 22451120 DOI: 10.1007/s10620-012-2136-1]

89 **Hagiwara S**, Kudo M, Nagai T, Inoue T, Ueshima K, Nishida N, Watanabe T, Sakurai T. Activation of JNK and high expression level of CD133 predict a poor response to sorafenib in hepatocellular carcinoma. *Br J Cancer* 2012; **106**: 1997-2003 [PMID: 22596232 DOI: 10.1038/bjc.2012.145]

90 **Cherqui D**, Laurent A, Mocellin N, Tayar C, Luciani A, Van Nhieu JT, Decaens T, Hurtova M, Memeo R, Mallat A, Duvoux C. Liver resection for transplantable hepatocellular carcinoma: long-term survival and role of secondary liver transplantation. *Ann Surg* 2009; **250**: 738-746 [PMID: 19801927 DOI: 10.1097/SLA.0b013e3181bd582b]

91 **Adam R**, Azoulay D, Castaing D, Eshkenazy R, Pascal G, Hashizume K, Samuel D, Bismuth H. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003; **238**: 508-18; discussion 518-9 [PMID: 14530722 DOI: 10.1097/01.sla.0000090449.87109.44]

92 **Fuks D**, Dokmak S, Paradis V, Diouf M, Durand F, Belghiti J. Benefit of initial resection of hepatocellular carcinoma followed by transplantation in case of recurrence: an intention-to-treat analysis. *Hepatology* 2012; **55**: 132-140 [PMID: 21932387 DOI: 10.1002/hep.24680]

P- Reviewer: Scherubl H **S- Editor**: Gou SX **L- Editor**: A
E- Editor: Zhang DN

WJG
World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7327

World J Gastroenterol 2013 November 14; 19(42): 7327-7340
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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REVIEW

Hepatobiliary manifestations in inflammatory bowel disease: The gut, the drugs and the liver

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Author contributions: Romero-Gómez M designed the concept for this review and contributed to the writing and reviewing of the final revision of the manuscript; Rojas-Feria M, Castro M, Ampuero J, and Suárez E contributed to the study design, literature search, manuscript writing, and final revision of the article; all authors approved the final version of the manuscript.

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Received: June 27, 2013 **Revised:** September 23, 2013
Accepted: September 29, 2013
Published online: November 14, 2013

Abstract

Abnormal liver biochemical tests are present in up to 30% of patients with inflammatory bowel disease (IBD), and therefore become a diagnostic challenge. Liver and biliary tract diseases are common extraintestinal manifestations for both Crohn's disease and ulcerative colitis (UC), and typically do not correlate with intestinal activity. Primary sclerosing cholangitis (PSC) is the most common hepatobiliary manifestation of IBD, and is more prevalent in UC. Approximately 5% of patients with UC develop PSC, with the prevalence reaching up to 90%. Cholangiocarcinoma and colon cancer risks are increased in these patients. Less common disorders include autoimmune hepatitis/PSC overlap syndrome, IgG4-associated cholangiopathy, primary biliary cirrhosis, hepatic amyloidosis, granulomatous hepatitis, cholelithiasis, portal vein thrombosis, liver abscess, and non-alcoholic fatty liver disease. Hepatitis B reactivation during immunosuppressive therapy is a major concern, with screening and vaccination being recommended in serologically negative cases for patients with IBD. Reactivation prophylaxis with entecavir or tenofovir for 6 to 12 mo after the end of immunosuppressive therapy is mandatory in patients showing as hepatitis B surface antigen (HBsAg) positive, independently from viral load. HBsAg negative and anti-HBc positive patients, with or without anti-HBs, should be closely monitored, measuring alanine aminotransferase and hepatitis B virus DNA within 12 mo after the end of therapy, and should be treated if the viral load increases. On the other hand, immunosuppressive therapy does not seem to promote reactivation of hepatitis C, and hepatitis C antiviral treatment does not influence IBD natural history either. Most of the drugs used for IBD treatment may induce hepatotoxicity, although the incidence of serious adverse events is low. Abnormalities in liver biochemical tests associated with aminosalicylates are uncommon and are usually not clinically relevant. Methotrexate-related hepatotoxicity has been described in 14% of patients with IBD, in a dose-dependent manner. Liver biopsy is not routinely recommended. Biologics-related hepatotoxicity is rare, but has been shown most frequently in patients treated with infliximab. Thiopurines have been associated with veno-occlusive disease, regenerative nodular hyperplasia, and liver peliosis. Routine liver biochemical tests are recommended, especially during the first month of treatment. All these conditions should be considered in IBD patients with clinical or biochemical features suggestive of hepatobiliary involvement. Diagnosis and management of these disorders usually involve hepatologists and gastroenterologists due to its complexity.

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Key words: Inflammatory bowel disease; Hepatobiliary disorders; Extraintestinal manifestations; Primary sclerosing cholangitis; Drug-induced liver injury; Hepatotoxicity; Hepatitis B; Hepatitis C

Core tip: Hepatobiliary disorders are common extrain-

testinal manifestations of inflammatory bowel disease (IBD) that become a diagnostic challenge for the gastroenterologist. In this review, we have summarized the main diseases involving the hepatobiliary system in IBD and secondary liver toxicity to IBD treatment. This review also highlights the impact of immunosuppressive and anti-tumor necrosis factor treatment in hepatitis B and C, as well as its prophylaxis and treatment, according to current clinical practice guidelines.

Rojas-Feria M, Castro M, Suárez E, Ampuero J, Romero-Gómez M. Hepatobiliary manifestations in inflammatory bowel disease: The gut, the drugs and the liver. *World J Gastroenterol* 2013; 19(42): 7327-7340 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7327.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7327

INTRODUCTION

Hepatobiliary diseases are relatively common in inflammatory bowel disease (IBD) and therefore become a diagnostic challenge. Liver and biliary tract disorders are typical extraintestinal manifestations in both Crohn's disease (CD) and ulcerative colitis (UC). In patients receiving immunosuppressive therapy, including biologics, the risk of hepatitis B reactivation is high, so patients undergoing this therapy should be screened for hepatitis B surface antigen (HBsAg) and anti-HBc prior to starting the treatment. Most of the drugs used for IBD treatment have also been associated with hepatotoxicity. All these conditions should be ruled out in IBD patients with clinical or biochemical features suggestive of liver involvement, as summarized in this review.

Bibliographic searches were performed in the MEDLINE electronic database up to February 2013 using the Medical Subject Headings terms: ("inflammatory bowel disease" OR "Crohn's disease" OR "ulcerative colitis") AND ("liver" OR "biliary tract" OR "primary sclerosing cholangitis" OR "hepatobiliary disorders" OR "small-duct PSC" OR "PSC/AIH overlap syndrome" OR "IgG4-associated cholangitis" OR "primary biliary cirrhosis" OR "hepatic amyloidosis" OR "granulomatous hepatitis" OR "cholelithiasis" OR "portal vein thrombosis" OR "liver abscess" OR "non-alcoholic fatty liver disease" OR "viral hepatitis" OR "hepatitis B" OR "hepatitis C" OR "drug-induced liver injury" OR "drug-induced hepatitis" OR "hepatotoxicity").

HEPATOBILIARY DISORDERS ASSOCIATED WITH INFLAMMATORY BOWEL DISEASE

Hepatobiliary manifestations constitute some of the most common extraintestinal manifestations of IBD. They typically adopt an independent course irrespective of intestinal activity and are present in both UC and CD.

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic fibroesclerotic disorder of the intrahepatic and extrahepatic biliary tree. and is the most common hepatobiliary manifestation of IBD. The association of PSC and IBD was described for first time in 1965[1].

Epidemiology: Approximately 70%-80% of patients with PSC have concomitant IBD and about 1.4%-7.5% of patients with IBD will develop PSC[2]; however the course of IBD is not related to PSC. It is more prevalent in males, in UC, and in young and middle-aged patients[3]. UC has been reported in 25 to 90 percent of patients with PSC[4]. In a Spanish multicenter study, based on a survey, UC was present in 44%[5]. Nevertheless, the real prevalence of UC in PSC is up to 90% when rectal and sigmoid biopsies are routinely obtained[6].

PSC is typically characterized by progressive inflammation, obliterative fibrosis, and destruction of intra- and extrahepatic bile ducts, leading to end-stage liver disease and portal hypertension[7]. Patients with PSC may also develop complications such as cholestasis-associated manifestations, biliary stricture, cholangitis, cholelithiasis, cholangiocarcinoma, and colon cancer. The diagnosis of PSC is usually previous to IBD, but PSC may be diagnosed over time, after a proctocolectomy in UC patients[8].

Etiology: The etiology of PSC remains unclear. Genetic, immunological, and environmental factors seem to contribute to its pathogenesis. First-degree relatives of patients with PSC show an increased risk of PSC and UC, supporting a genetic predisposition to these conditions[9]. Multiple genetic factors associated with susceptibility have been described, like HLA-B8, HLA-DRB1*0301 (DR3), HLADRB3*0101 (DRw52a), and HLA-DRB1*0401 (DR4)[10,11]. In addition, three UC susceptibility loci have been associated with PSC, harboring the presumed candidate genes REL, IL2, and CARD9[12]. An autoimmune mechanism has been suggested, since both are immune-mediated disorders, and are also associated with other autoimmune diseases. Several autoantibodies may be present, such as antinuclear antibodies (ANA) in 24%-53%, smooth muscle antibodies (SMA) in 13%-20%, and anti-perinuclear cytoplasmic antibodies (pANCA) in 65%-88% of patients[13-15]. Other autoantibodies, including anticardiolipin, thyroperoxidase, and rheumatoid factor may be present, but show uncertain clinical significance. In one study, 97% of cases with PSC were positive for, at least, one autoantibody, while 81% were positive for three or more[16]. An inflammatory response to chronic or recurrent bacterial infection into the portal circulation or ischemic damage to the bile ducts has also been postulated[17]. Therefore, the most plausible theory involves the exposure of genetically predisposed individuals to an environmental agent that provokes an anomalous immune response, leading to disease development.

IBD in PSC patients has a distinct behavior, as it shows a higher incidence of rectal sparing, backwash ileitis, extensive colitis, pouchitis after ileal pouch anal anastomosis, colon dysplasia, colon cancer, and poorer prognosis[18-21]. Patients with UC and PSC usually have a lower grade of colon inflammation and a milder course, compared to patients without PSC[22]. In addition, severe progressive PSC requiring liver transplantation appears to reduce histological activity and the need for colectomy in UC[23,24].

Diagnosis: Most patients with PSC are asymptomatic at diagnosis. This disease should be considered in patients with IBD and abnormal liver biochemical tests, where a marked elevation of serum alkaline phosphatase is commonly found[25]. In symptomatic patients, fatigue and pruritus are common. Other features include abdominal pain, jaundice, and weight loss. Cholangitis occurs in 10%-15% of patients during the course of the disease. Biochemical tests usually show a cholestatic pattern. Aminotransferases levels are typically lower than 300 IU/L. Additional biochemical parameters are hypergammaglobulinemia (30% of cases), increased serum IgM levels (40%-50%), and p-ANCA (30%-80%). Serum albumin levels later decrease during the course of the disease, and the presence of hypoalbuminemia earlier may indicate active IBD.

Diagnosis is established by the demonstration of diffuse, multifocal strictures and dilations in the intra- and extrahepatic bile ducts. In 41% of cases, the gallbladder and cystic duct may also be involved[26]. In the early stages of the disease, superficial ulcerations of the bile ducts may be the only manifestation found. Endoscopic retrograde cholangiopancreatography (ERCP) is considered the gold standard technique for PSC diagnosis. It can be both diagnostic and therapeutic, and also may be useful in the early diagnosis of cholangiocarcinoma. Magnetic resonance cholangiography (MRCP) is a non-invasive alternative with high sensitivity and specificity, and without the risks related to the technique[27]. Liver biopsy is only recommended in cases of clinical suspicion of small-duct PSC, as it is rarely diagnostic of PSC[28]. The most specific histologic finding in PSC is fibrous obliteration of small bile ducts, with periductal concentric fibrosis in an “onion skin” pattern. Other abnormalities are non-specific and similar to those in primary biliary cirrhosis. Liver biopsy is helpful for staging the disease and determining prognosis. Ludwig described 4 stages of PSC based on morphologic features[29].

Prognosis: PSC is a progressive disease that, ultimately, results in portal hypertension, cirrhosis, and hepatic failure. The median survival time without liver transplantation is approximately 12 years. Survival is significantly worse in symptomatic patients at the time of diagnosis[30]. Coexisting IBD may also be related to a poorer prognosis, as it has been associated with a younger age at diagnosis, the development of malignant complications, dysplasia and/or colon cancer[31,32]. Patients with PSC usually develop complications of end-stage liver disease with portal hypertension, such as varices, ascites, and hepatic encephalopathy. The Mayo Risk Score based on age, serum bilirubin, albumin, aspartate aminotransferase, and the presence of variceal bleeding, has been used to assess disease progression and prognosis[33]. Other complications include steatorrhea and fat-soluble vitamin deficiency, secondary to chronic cholestasis, amyloidosis secondary to amyloid A protein deposition in tissues due to a progressive inflammatory process[34], dominant biliary strictures, cholangiocarcinoma, and colon cancer. The risk for cholangiocarcinoma is significantly increased in PSC and its development remains unpredictable. The annual incidence has been estimated as 1.5%[35]. Risk factors include the presence of IBD, cirrhosis, variceal bleeding, a dominant stricture in the bile duct, and alcohol intake[36]. Worsening jaundice, weight loss, and abdominal discomfort are suspicion symptoms. Diagnosis may be difficult, as imaging techniques and brush cytology show a lack of sensitivity for early detection. However, ERCP and cytology of bile duct strictures is highly specific[37]. Prognosis is devastating, with a survival rate of 10% two years after diagnosis[38] and a recurrence rate in the transplanted liver of about 20%-25%[39]. Patients with PSC have an increased risk for gallbladder cancer, pancreatic cancer and, in cirrhotic patients, hepatocellular carcinoma. A higher risk of colorectal dysplasia/cancer has also been described among UC patients with PSC[21], even after liver transplantation[40]. The severity and the duration of PSC have not been significantly associated with the risk of colon cancer[41]. In patients with ileal pouch-anal anastomosis, the risk for dysplasia persists after colectomy[42]. Therefore, surveillance for colorectal cancer should be strongly recommended in PSC patients with UC[43].

Treatment: Treatment of PSC associated with UC does not differ from PSC without IBD. As no pharmacologic therapy has proven effective for PSC, treatment goals are the control of symptoms and the management of complications. Ursodeoxycholic acid (UDCA) has been shown as effective in liver function improvement based on biochemical tests but it had no effect on liver histology, liver transplant-free survival, requirements for liver transplantation, development of cholangiocarcinoma, or incidence of death[44,45]. In a meta-analysis, UDCA does not appear to decrease either the risk of adenomas or colon cancer[46]. Immunosuppressants, chelators, and steroids have been used without any benefit.

Liver transplantation is the only therapy that can change the inevitable outcome. The appropriate moment for liver transplantation can be difficult to determine, as patients with advanced disease may not show signs of liver failure. Survival rates after hepatic transplant at 5 and 10 years are 85% and 70%, respectively[47]. However, in 20%-25% of cases, PSC recurs in the transplanted liver[39].

Endoscopic management of PSC is indicated in cases of cholangitis, exacerbate jaundice, or suspicion of cholangiocarcinoma. Endoscopic dilation of dominant strictures, with or without stenting, has been shown to alleviate cholestasis and to improve laboratory test results, although it does not prevent disease progression[48].

Small-duct PSC

Small-duct PSC is characterized by laboratory and histological findings similar to PSC but with normal cholangiogram. The presence of coexisting IBD is required for the diagnosis of this entity[49]. In a large multicenter study, 80% of patients with small-duct PSC had concurrent IBD (78% UC and 21% CD)[50]. Progression of small-duct PSC to PSC was observed in 12%-23% of cases. Small-duct PSC has been associated with a better long-term prognosis as compared with large-duct PSC. Cholangiocarcinoma has not been previously described. Some patients may require liver transplantation for end-stage liver disease, and the disease may recur after liver transplantation. In IBD patients with cholestatic liver function tests altered and a normal cholangiogram by ERCP/MRCP, a biopsy is recommended to rule out small-duct PSC, after excluding other hepatobiliary disorders.

AIH/PSC overlap syndrome: AIH/PSC overlap syndrome has been described in patients with IBD, especially UC[51]. The diagnosis is suspected when features of AIH and PSC are present in the same patient, requiring a definitive diagnosis of AIH based on the International Autoimmune Hepatitis Group Criteria, which includes demographic, histologic, and laboratory markers[52]. Diagnosis, treatment, and prognosis of the overlap syndrome are controversial and so standardized diagnostic criteria are needed[53,54]. Previous studies have reported cases that initially presented with laboratory markers and histologic features of either AIH or both diseases with a normal cholangiography, only to develop pathologic characteristics of PSC during the follow-up[52,55,56]. Intrahepatic and extrahepatic bile ducts may be affected. Conventional corticosteroid therapy, alone or in conjunction with UDCA (13-15 mg/kg daily), has been variably effective, and cyclosporine, mycophenolate mofetil, and budesonide have been beneficial in selected patients. The cholestatic features that influence the prognosis of autoimmune hepatitis must be defined and incorporated into the definition of the syndrome[57].

IgG4-associated cholangiopathy

IgG4-associated cholangiopathy (IAC) is a biliary disease of unknown immunopathogenesis. Indistinguishable from PSC according to cholangiographic characteristics, it shows distinct histological findings. It is one of a variety of IgG4-related systemic disease and has been described in patients with concurrent UC[58]. Clinical diagnostic criteria for IgG4-related disease require systemic organ involvement, elevated serum IgG4 levels ($\geq$ 135 mg/dL), and histopathological findings[59]. IgG4 levels have also been reported in 9%-36% of patients with PSC, although these levels are usually lower than in patients with IAC[60,61]. The identification of IgG4 plasma cell infiltrating the bile duct and other organs is decisive in reaching the diagnosis[58,59]. Clinically, patients with IAC are older at diagnosis compared to patients with PSC. Obstructive jaundice can be the first symptom, whereas it is rarely present in PSC[62]. Steroids are the first-choice therapy of IAC, as they result in the resolution of jaundice, improve liver laboratory parameters, and reduce serum IgG4 levels and the reversal of strictures on cholangiogram[63]. Azathioprine (AZA) should be considered alongside those with proximal and intrahepatic stenosis, and those that relapse during and/or after corticosteroid therapy[64].

Primary biliary cirrhosis

Primary biliary cirrhosis (PBC) frequently accompanies various autoimmune diseases including Sjögren syndrome, chronic thyroiditis, and rheumatoid arthritis, but rarely IBD[65]. There are a few reported cases of both diseases in the literature[66,67]. The clinical presentation varies from typical PBC; affecting males more frequently, being diagnosed at younger age and at earlier stages of PBC, and usually associated with previously diagnosed mild left-side UC. Although the pathogenesis of this disease has not yet been clarified, environmental and genetic factors are considered important in the susceptibility to both diseases.

Hepatic amyloidosis

Secondary amyloidosis is an unusual complication of IBD, more frequent in CD than in UC (0.9% *vs* 0.07%)[68]. Chronic activity in the bowel contributes to amyloid deposition in the vasculatures and sinusoids of almost any organ, including the liver. It may present as asymptomatic hepatomegaly and is more common in men with colonic diseases. Treatment is based on controlling gut inflammation, thereby decreasing the release of the acute phase reactant serum amyloid A[69]. In some cases, colchicine can be effective.

Granulomatous hepatitis

Granulomatous hepatitis is another rare complication of CD, which is characterized by granulomas on the liver biopsy. The main manifestation is an increase in cholestatic enzymes such as alkaline phosphatase. Granulomatous hepatitis is often secondary to different medications, including sulfasalazine[70]. Other causes are malignancies, infections, or CD metastasis[71]. Corticosteroids and immunosuppressive drugs have been used in its treatment.

Cholelithiasis

It has been estimated that patients with CD have a doubled risk for gallstones comparing to IBD-free controls, while UC is not associated with an increased risk[72]. The incidence of gallstones is raised in patients with Crohn's ileitis or ileal resection, ranging from 13% to 34%[73]. Risk factors associated with its development are CD location at diagnosis, surgery, and extent of ileal resection. Other factors include the age of the patient, frequency of clinical recurrences, length of hospital stay, and the use of total parenteral nutrition. The pathophysiology of cholelithiasis in CD is not well defined. Abnormal malabsorption of bile acids that interfere with enterohepatic circulation has been proposed. Moreover, reduced gallbladder motility has been described in CD and increased gallstone cholesterol concentrations have been identified in patients with ileoanal anastomosis[74].

Portal vein thrombosis

IBD is associated with an increased risk of vascular complications, such as arterial and venous thromboembolisms, which are considered extraintestinal manifestations. Portal vein thrombosis is a rare but potentially life-threatening complication, with an incidence in IBD patients higher than that of the general population. In a Mayo Clinic study, portal/mesenteric vein thrombosis was reported in 1.3% of IBD cases, with a mortality of 50%[75]. Recent abdominal surgery, younger age, and female gender are associated with a higher incidence of portal vein thrombosis[76]. The factors involved in this pathogenesis are diverse. Acquired prothrombotic factors can be identified, such as inflammation, immobilization, extent of colon disease, surgery, central catheters, corticosteroids, and smoking[77,78]. Furthermore, patients with IBD have increased platelet counts, factor Ⅴ and Ⅷ levels, and fibrinogen, along with decreased antithrombin Ⅲ levels. Anticoagulants, such as low-molecular-weight heparin and warfarin, are mainstays of therapy, even in the setting of gastrointestinal bleeding[73]. In the presence of a congenital hypercoagulable state, lifelong systemic anticoagulation should be considered, although in other prothrombotic conditions, a six-month course provides adequate coverage[79].

Liver abscess

The association between liver abscesses and IBD is uncommon[80,81], but hepatic abscesses can be an initial manifestation of CD[82]. The mechanism of abscess development may be related to direct extension of intra-abdominal abscesses or due to portal pyemia, secondary to an increase in intestinal mucosa permeability. Among associated risk factors, intra-abdominal abscesses, fistulizing disease, malnutrition, and treatment with steroids and metronidazole have been reported.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a clinic-pathological syndrome with a histologic spectrum ranging from benign steatosis to non-alcoholic steatohepatitis (NASH)[83]. Steatosis is described in up to 50% of abnormal liver biopsies in IBD patients and has been related to colitis severity. It was presumed secondary to severe illness, with malnutrition, hypoproteinemia, and corticosteroids primarily responsible[84]. On the other hand, NAFLD occurs in 8.2% of the IBD population, which is much lower than the frequency reported in the United States general population (33.6%). Those patients who developed NAFLD tended to be older, and developing IBD at an older age normally requires small bowel surgery[85]. It has been reported that IBD patients develop NAFLD with fewer metabolic risk factors than non-IBD NAFLD patients. In multivariate analysis, hypertension (OR = 3.5), obesity (OR = 2.1), small bowel surgeries (OR = 3.7), and use of steroids at the time of imaging (OR = 3.7) were independent factors associated with NAFLD. NAFLD is also less common among patients who received antibodies against tumor necrosis factor alpha (anti-TNF-α) therapy.

VIRAL HEPATITIS AND IBD

Chronic hepatitis B and C are two common diseases. The WHO estimates that 350 million people in the world suffer from chronic hepatitis B, and more than 200 million from hepatitis C[86,87]. Hepatitis B infection is transmitted during delivery or early childhood. Hepatitis C is a blood-borne disease spread mainly *via* blood product transfusion and misuse of illegal drugs. Around 20% of patients with chronic hepatitis B show cirrhosis progression and 5% are at risk of developing hepatocellular carcinoma.

Chronic hepatitis B infection is a dynamic process, owing to the interaction between the hepatitis B virus and the host immune system. Its natural history is covered by five different phases[88] (Table 1): (1) Immunotolerant phase: characterized by positive hepatitis B e antigen (HBeAg), higher HBVDNA titre, and normal or near normal ALT levels. Liver biopsy at this phase shows mild or no inflammatory lesions and scarce non-progressive fibrosis; (2) Immunoclearance phase: positive HBeAg, lower HBVDNA, and raised aminotransferase levels. Liver histology shows necroinflammatory activity together with fibrosis progression. Spontaneous HBeAg clearance and anti-HBe seroconversion could occur at this phase; (3) HBsAg inactive carrier: characterized by negative HBeAg, positive anti-HBe, residual viremia (HBVDNA lower than 2000 IU/mL), and ALT levels under the normal limit. Liver histology shows minimal or no lesions; (4) Chronic hepatitis B: HBeAg negative could appear after unsuccessful seroconversion. HBVDNA remains quantifiable and ALT levels are fluctuant. Fibrosis progression is common; and (5) Resolved infection: characterized by loss of HBsAg and could be a stable phase, with negative HBsAg and non-detectable HBVDNA with normal ALT levels and excellent prognosis.

Chronic hepatitis C is a progressive liver disease that could evolve to cirrhosis. Risk factors associated with fibrosis progression are alcohol consumption, HIV co-infection, and adult age at infection.

Chronic hepatitis B patients showing HBVDNA > 2000 IU/mL and at least moderate necroinflammatory activity or fibrosis in a liver biopsy should be treated with entecavir or tenofovir and, in selected cases, with peginterferon α-2a. In patients with chronic hepatitis C and positive HCVRNA, antiviral treatment should be started. In genotype 1, protease inhibitor-based triple therapy is

Table 1 Phases of chronic hepatitis B infection

Phase	Characteristics	HBVDNA	ALT	Liver histology
Immune tolerant	HBeAg positive	> 2000000 UI/mL	Normal	Normal or mild inflammation
Immune active	HBeAg positive	> 200000 UI/mL	Elevated	Chronic hepatitis Active cirrhosis
Inactive carrier	HBeAg negative Anti-HBe positive	< 2000 UI/mL	Normal	Normal or mild inflammation Mild fibrosis Inactive cirrhosis
HBeAg negative chronic hepatitis	HBeAg negative Anti-HBe positive	> 20000 UI/mL	Elevated (fluctuating)	Chronic hepatitis Active cirrhosis
Remission	HBsAg negative Anti-HBc positive	Indetectable in serum and detectable in liver	Normal	Normal Mild fibrosis Inactive cirrhosis

HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen.

the first choice and, in non-1 genotype, standard peginterferon plus ribavirin.

Viral reactivation during immunosuppressive therapy is a major concern in viral hepatitis B and C. Viral reactivation is defined by an increase of 1 log in viral load or re-appearance of the virus after previous clearance. A flare of ALT is common and in some cases may develop into acute liver failure.

Hepatitis B reactivation depends on two main factors: (1) type of immunosuppressive drug used, and (2) hepatitis B phase prior to treatment. In a recent meta-analysis including 14 studies and 485 HBsAg-positive patients undergoing chemotherapy, one in three patients developed hepatitis and the mortality rate reached 7%. Positive HBeAg and detectable HBVDNA, together with steroids or rituximab in hematologic neoplasms, were independent factors associated with the risk of developing reactivation[89]. In patients with HBsAg loss, reactivation risk was around 3%-10% and mainly associated with the combination of steroids and rituximab in hematologic neoplasms[90].

Hepatitis B reactivation prophylaxis is recommended from one week before chemotherapy to 12 mo after cessation of this therapy. Lamivudine has shown to decrease mortality rate[89]. However, it has been associated with an increased risk of developing resistant variants, so drugs with a higher genetic barrier, like entecavir or tenofovir[91], are recommended if immunosuppressors need to be used for more than one year.

Hepatitis C reactivation management is controversial, due to both its frequency and clinical manifestations remaining unclear. Hepatitis has been reported in 11% of cases and reactivation in 36% of the few patients with HCVRNA evaluated before and after chemotherapy, mainly in those treated with rituximab for hematologic neoplasms[92]. Interferon-based therapy is not recommended for hepatitis C reactivation prophylaxis. Further combination of direct antiviral drugs could be useful in avoiding hepatitis C reactivation in this setting.

As immunosuppressive drugs, like AZA, methotrexate (MTX), and anti-tumor necrosis factor α (TNFα), are being used more frequently in IBD, concerns about viral reactivation are increasing.

Hepatitis B and IBD

At the beginning of this century, hepatitis B infection prevalence was slightly higher in patients with IBD than in the general population, which was mainly related to increased surgical procedures and blood transfusions[93,94]. However, recent studies in France and Spain did not confirm these data. Prevalence of hepatitis B in IBD has been estimated to be similar to the general population as a consequence of several health system measures, like overall vaccination, safe transfusions, and surgical procedures. Prevalence of anti-HBc was 7.1% in CD and 8% in UC in a Spanish population[95,96].

Hepatitis B reactivation is a major health problem. Patients undergoing immunosuppressive therapy are at risk of developing hepatitis B reactivation. Acute liver failure requiring orthotopic liver transplantation has been reported in a patient under AZA and steroid treatment[97]. TNFα plays a role in hepatitis B virus replication, so anti-TNFα drugs could promote hepatitis B reactivation[98]. Infliximab, together with AZA or steroids, have been implicated in hepatitis B reactivation in seven cases from several series[94,99-102]. No cases have been reported in patients receiving adalimumab or certolizumab pegol. Nevertheless, hepatitis B reactivation seems to be a class-effect, so more cases should be expected with the continuing use of these new drugs. In patients with HBsAg loss reactivation is infrequent, but one case treated with infliximab has been reported[103]. A collaborative multicenter and retrospective Spanish study (REPENTINA) showed a reactivation rate of 36% (9/25) in patients with positive HBsAg; six out of nine developed liver failure, three underwent liver transplantation, and one died. No HBsAg-negative patients developed hepatitis B virus reactivation. In this study, the key factor for reactivation was the combination of two or more immunosuppressive drugs, independently of agent type. The absence of reactivation was associated with the use of only one drug for a short period of time[104].

Clinical practice guidelines from AEEH, EASL, and ECCO revised this topic and recommended prophylaxis of hepatitis B reactivation in patients with IBD receiving immunosuppressive agents[104-106]. HBsAg, anti-HBc, and anti-HBs should be tested before immunosuppressive therapy with two goals: avoid possible fatal complications and use antiviral drugs to control hepatitis B virus replication: (1) HBV serologically-negative patients should receive vaccination. Vaccination rates in IBD are variable. In Spain, only 56% of young people showed anti-HBs antibodies[95]. Vaccine response was around 46% using double doses in patients with IBD receiving anti-TNF drugs. However, AZA seems not to influence vaccine response. In non-responders a new complete vaccination course should be recommended. Hepatitis B screening is highly recommended immediately after the diagnosis of IBD, and vaccination is indicated in serologically-negative patients[107]; (2) Patients showing HBsAg positive and HBVDNA > 2000 IU/mL should be treated with tenofovir or entecavir as chronic hepatitis B patients; (3) Patients showing HBsAg positive and HBVDNA < 2000 IU/mL or undetectable levels, and patients with HBsAg negative and HBVDNA positive should be treated with tenofovir or entecavir for 6 to 12 mo after the end of immunosuppressive therapy. ALT levels and HBVDNA titre should be monitored every three months during treatment; and (4) Patients showing HBsAg negative and anti-HBc positive with or without anti-HBs should be closely monitored every 1 to 3 mo, measuring ALT and HBVDNA until 6 to 12 mo after the end of therapy. For patients with an increase in viral load, entecavir or tenofovir therapy should be immediately started.

Hepatitis C and IBD

The prevalence of hepatitis C infection was increased in IBD patients younger than 50-year-old in comparison with the general population in studies of the last decade of the twentieth century[94]. However, recent studies have demonstrated a similar prevalence to the general population both in Spain (2.3% in CD and 1.3% in UC) and France (0.79% in CD and 1.59% in UC)[96,97].

The impact of immunosuppressive therapy for IBD on hepatitis C remains controversial. Steroids could promote viral replication, as demonstrated after liver transplantation. However, steroids have been used to treat hepatitis C without success or adverse events. ALT flares have been reported after stopping steroid therapy in patients with IBD[108]. Steroid therapy should be avoided in patients with hepatitis C, and patients should be closely monitored after any withdrawal or tapering process. Immunosuppressive drugs like AZA, MTX, cyclosporine, and mycophenolate mofetil have been largely used in the liver transplantation setting, showing a slight antiviral activity against hepatitis C[109-111]. MTX did not affect hepatitis C disease in patients with arthritis[112]. Therefore, immunosuppressive therapy with these drugs seems to be safe. Indeed, in a Spanish study, 16% (8/51) of patients with chronic hepatitis C and IBD receiving immunosuppressive therapy developed non-severe liver dysfunction, seven related to steroids, and one case with AZA[104].

TNFα plays a major role in the pathogenesis of chronic hepatitis C and associated metabolic abnormalities. TNFα is crucial in hepatitis C-induced insulin resistance, but could also modulate interferon response. Increased TNFα levels have been associated with impaired sustained virological response (SVR)[113]. Thus, TNFα inhibition could be more beneficial than harmful in the management of chronic hepatitis C. Etanercept improved SVR in patients with chronic hepatitis C receiving peginterferon plus ribavirin[114]. Patients suffering from rheumatoid arthritis and hepatitis C treated with etanercept or infliximab did not show any changes in transaminases level or viral load[115]. Although etanercept is not effective on IBD, a class-effect should be expected, and the use of anti-TNFα could be safe in hepatitis C associated with IBD.

CD is characterized by a Th1 response. Interferon seems to play an immunomodulatory activity-enhancing Th1 response, and can cause outbreak of CD[116]. On the other hand, some authors have suggested that interferon could be safely used in patients with IBD, due to a lack of negative impact on the gut[117,118]. In a prospective study including 11 patients with IBD and hepatitis C, peginterferon plus ribavirin achieved SVR in a similar way to non-IBD patients. During treatment six patients developed gastrointestinal symptoms that required optimization of immunosuppressive agents without impacting antiviral treatment[119]. Several case reports showed a beneficial effect of interferon-alpha and beta on patients with UC[120,121]. A systematic review, including three prospective studies, demonstrated no effect of interferon-alpha on UC[122], although new cases or exacerbation of UC have been seen in patients treated with interferon-alpha[123,124]. Higher doses of interferon used in hepatitis C, in comparison with lower doses in UC trials, could partially explain this discrepancy. Finally, 10 patients with CD and 10 with UC in remission or showing mild bowel activity underwent antiviral therapy for hepatitis C. No patient developed reactivation of IBD during treatment or the 12 mo of follow-up[125], confirming data from a recent review concluding that interferon does not impair IBD course[126].

In summary, hepatitis C antiviral treatment has no influence on IBD natural history, and immunosuppressive therapy for IBD does not promote reactivation of hepatitis C. Currently, no therapeutic option for reactivation prophylaxis nor vaccination are available for hepatitis C, but in the near future interferon-free regimen combining protease, polymerase, and NS5A inhibitors could be useful in the management of hepatitis C in IBD.

DRUG-INDUCED LIVER INJURY IN IBD

Approximately 30% of patients with IBD show abnormalities in liver biochemical tests during the course of the disease. Most of the drugs used in IBD have potential hepatotoxicity[127] (Table 2).

Aminosalicylates: Sulfasalazine and mesalazine

Sulfasalazine, an association between sulfapyridine and 5-aminosalicylate (5-ASA), was the first aminosalicylate used in the treatment of IBD. This drug has been replaced in the last two decades by mesalazine or 5-ASA,

Table 2 Drug induced hepatobiliary manifestations in inflammatory bowel disease

Manifestation	Drug
Drug induced hepatitis	Azathioprine 6-mercaptopurine Methotrexate Cyclosporine Infliximab
Reactivation of hepatitis B	Anti-TNF therapy Corticosteroids
Drug induced pancreatitis	Azathioprine 6-mercaptopurine Methotrexate
Hepatosplenic T-cell lymphoma	Combination of anti-TNF and immunosuppressive therapy

TNF: Tumor necrosis factor.

due to its adverse effects. Mesalazine is indicated for the induction and maintenance of the clinical remission in patients with UC with mild-moderate activity. The efficacy of this drug in CD remains controversial.

The anti-inflammatory effect of aminosalicylates is unclear. Synthesis inhibition of prostaglandins and leukotrienes (which show antioxidant and immunomodulatory activity) are well known. Aminosalicylates are safe drugs, and rarely lead to severe adverse effects such as bone marrow aplasia, pancreatitis, nephropathy, or hepatotoxicity. Altered liver function tests (cytolysis or cholestasis) may be detected during treatment with them. These abnormalities usually have no clinical relevance, although hepatotoxicity induced by acute hypersensitivity and acute liver failure has been described. In clinical trials, abnormalities in liver biochemical tests have been observed in 2% of UC patients treated with mesalazine[128]. The United Kingdom's Committee on Safety of Medicines observed that, between 1991 and 1998, the incidence of toxic hepatitis was 3.2 and 6 cases per million of prescriptions for mesalazine and sulfasalazine, respectively, and the presence of rheumatoid arthritis was a stronger risk factor than IBD[129]. Therefore, given the low risk of hepatotoxicity, a close monitoring of liver biochemical tests is not necessary in patients treated with aminosalicylates.

Methotrexate

Methotrexate (MTX) has both anti-proliferative and immunosuppressive activities, impairing DNA synthesis (*via* inhibition of dihydrofolate reductase) as well as decreasing the production of proinflammatory cytokines and inducing lymphocyte apoptosis, respectively. The main indication in IBD is maintenance of clinical remission in steroid-dependent CD patients, after adverse effects or lack of efficacy of thiopurines. MTX efficacy in UC is controversial. On the other hand, MTX is contraindicated in pregnancy. Regarding adverse effects, myelosuppression and hepatotoxicity are dose-dependent. These effects were documented in up to 25% of patients with rheumatoid psoriatic arthritis, highlighting the association with obesity, alcoholism, diabetes, previous abnormalities in biochemical liver tests and, especially, an accumulated dose higher than 15 g, as risk factors. Currently, liver fibrosis and cirrhosis are less frequent, probably due to close monitoring of liver parameters, proper selection of patients, and simultaneous treatment with folic acid, which decreases MTX-related adverse effects. Sabeni *et al*[130], in Italy, detected hepatotoxicity in 14.3% of patients with IBD treated with MTX during a mean follow-up of 26 mo. Te *et al*[131], in the United States, carried out a study in 20 IBD-patients treated with MTX with a mean follow-up of 131 mo and accumulated doses of 2.6 g. Liver fibrosis in biopsies was detected in one patient; the rest of the patients showed mild histological changes only. No association between abnormalities in liver biochemical tests and liver histology was found[131]. Regular liver laboratory studies are recommended in patients treated with MTX[132]. Nowadays, liver biopsy is not recommended routinely during MTX treatment[133]. However, it should be performed in cases of persistent alteration of transaminases (especially if they do not decrease after reducing the drug dose) and in patients with high accumulated doses, together with other risk factors. On the other hand, transient elastography (Fibroscan®) is emerging as diagnostic method of liver fibrosis in these patients. Treatment needs to be discontinued in cases of liver fibrosis or cirrhosis[134].

Anti-TNFα: Infliximab and adalimumab

Infliximab and adalimumab are monoclonal antibodies against TNFα, indicated in several rheumatologic, dermatologic, and gastrointestinal diseases. The main indication is the induction and maintenance of clinical remission in steroid-resistant or steroid-dependent CD and UC patients without response to immunosuppressive therapy. Early on, they are recommended in CD associated with risk factors or in the presence of severe perianal disease. Biological agents inhibit TNFα, preventing the release of proinflammatory cytokines, leukocyte migration, expression of endothelial molecules, fibroblast proliferation, and prostaglandin synthesis.

The main adverse effects of these drugs are opportunistic infections, hepatitis B virus reactivation, lymphoproliferative diseases, neurological diseases, and autoimmune diseases, such as lupus-like syndromes. Severe hepatotoxicity is a very rare condition in biological therapy, being most frequent in patients treated with infliximab. It is difficult to determine cause-effect associations between liver damage and these drugs in some cases due to confounding factors, like the presence of other drugs and other concomitant diseases. The drug label of infliximab (Remicade®) showed that, in clinical trials, ALT raised more than three times in 4.9% of patients with CD and in 2.5% of patients with UC, without clinical relevance. In contrast, serum ALT levels were less altered in the placebo group. On the other hand, the drug label of adalimumab (Humira®) indicates that serum ALT levels were similar in patients with IBD and a placebo.

According to infliximab indications, jaundice has been an uncommon finding, as well as infectious hepatitis, with liver failure being a very rare condition[135]. The Food and Drug Administration considers infliximab a hepatotoxic drug[136]. Recently, hepatotoxicity by these drugs has been evaluated in the United States (2003-2011), where only 34 cases were found, confirming the peculiarity of this adverse effect. Most cases (76%) were related to infliximab, showing a hepatocellular or cholestatic pattern with autoimmune characteristics, and improving after discontinuation of the drug[137]. Cross hepatotoxicity has not been documented in anti-TNF agents. In fact, in cases of infliximab-induced hepatotoxicity, adalimumab has been shown to be safe[138].

Thiopurines (azathioprine and 6-mercaptopurine)

AZA and its metabolite, 6-mercaptopurine (MP), are the immunosuppressive agents most commonly used in IBD. They are purine analogues, which interfere in nucleic acid synthesis and inhibit the proliferation of B and T lymphocytes, although the most relevant action is the apoptotic activation of T lymphocytes. The main indication of these drugs in CD and UC is the maintenance of clinical remission, preventing the use of steroids.

The active metabolites of AZA and MP are the 6-thioguanine nucleotides. In the liver, AZA is modified to MP, which is metabolized by xanthine oxidase and thiopurine methyltransferase (TPMT) in 6-thiouric acid and 6-methylmercaptopurine, resulting ultimately in 6-thioguanine nucleotides by hypoxanthine phosphoribosyltransferase. The decreased activity of TPMT facilitates the increasing in 6-thioguanine nucleotide levels, which are related to adverse effects. In fact, the efficacy of AZA and MP is limited owing to their adverse effects, which are responsible for treatment discontinuations in up to 15% of patients. Adverse effects are classified as dose-independent, dose-dependent, or idiosyncratic (which appears during the first two weeks of treatment). Regarding dose-independent adverse effects, the most common are allergic reactions (fever, exanthema, myalgias, and arthralgias) and acute pancreatitis. Among dose-dependent adverse effects, gastrointestinal intolerance and myelotoxicity are present in 2%-5% of patients. In retrospective studies, hepatotoxicity affected 3% of patients with an annual incidence of 1.4%, while these results are higher in prospective studies (10%)[139]. AZA and MP are able to damage the vascular endothelium, especially sinusoids and terminal veins, promoting veno-occlusive disease, regenerative nodular hyperplasia, and liver peliosis. These complications could be detected between 3 mo and 3 years after the beginning of treatment, and generate portal hypertension[140,141]. In general, mechanisms for AZA and MP hepatotoxicity remain unclear. It is thought that the main reason is the intracellular accumulation of 6-thioguanine nucleotides due to the decreased activity of TPMT.

It is recommended to determine levels of TPMT before the beginning of treatment with AZA or MP and routinely perform liver biochemical tests, especially during the first months of treatment, to detect myelotoxicity and/or hepatotoxicity. Mild abnormalities in liver parameters, without clinical relevance, allow the continuation of treatment at a lower dose. However, jaundice or persistent alterations in spite of reduced dose require an immediate stop to treatment[142].

CONCLUSION

Hepatobiliary disorders are common extraintestinal manifestations of IBD, and PSC represents the most prevalent disease among them. Abnormal liver biochemical tests are present in up to 30 percent of patients with IBD and emerge as a diagnostic challenge. Drug-induced hepatotoxicity should always be rule out, as most IBD treatments have been associated with liver toxicity, although the incidence of serious complications is low. Hepatitis B screening and vaccination is recommended in patients with IBD. Reactivation prophylaxis with entecavir or tenofovir is mandatory in patients under immunosuppressive therapy showing HBsAg positive, independently from viral load. HBsAg negative and anti-HBc positive patients, with or without anti-HBs, should be closely monitored, and treated if the viral load increase. Diagnosis complexity often requires a joint gastroenterologist and hepatologist approach.

REFERENCES

1 **Smith MP**, Loe RH. Sclerosing cholangitis; review of recent case reports and associated diseases and four new cases. *Am J Surg* 1965; **110**: 239-246 [PMID: 14313190 DOI: 10.1016/0002-9610(65)90018-8]

2 **Broomé U**, Bergquist A. Primary sclerosing cholangitis, inflammatory bowel disease, and colon cancer. *Semin Liver Dis* 2006; **26**: 31-41 [PMID: 16496231 DOI: 10.1055/s-2006-933561]

3 **Wiesner RH**, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, Fleming TR, Fisher LD, Beaver SJ, LaRusso NF. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. *Hepatology* 1989; **10**: 430-436 [PMID: 2777204 DOI: 10.1002/hep.1840100406]

4 **Bambha K**, Kim WR, Talwalkar J, Torgerson H, Benson JT, Therneau TM, Loftus EV, Yawn BP, Dickson ER, Melton LJ. Incidence, clinical spectrum, and outcomes of primary sclerosing cholangitis in a United States community. *Gastroenterology* 2003; **125**: 1364-1369 [PMID: 14598252 DOI: 10.1016/j.gastro.2003.07.011]

5 **Escorsell A**, Parés A, Rodés J, Solís-Herruzo JA, Miras M, de la Morena E. Epidemiology of primary sclerosing cholangitis in Spain. Spanish Association for the Study of the Liver. *J Hepatol* 1994; **21**: 787-791 [PMID: 7890895 DOI: 10.1016/S0168-8278(94)80240-8]

6 **Tung BY**, Brentnall TA, Kowdley KV, Emond M, Kimmey MB, Stevens AC, Rubin CE, Haggitt RC. Diagnosis and prevalence of ulcerative colitis in patients with sclerosing cholangitis (abstract). *Hepatology* 1996; **24**: 169A

7 **Lee YM**, Kaplan MM. Primary sclerosing cholangitis. *N Engl J Med* 1995; **332**: 924-933 [PMID: 7877651 DOI: 10.1056/NEJM199504063321406]

8 **Joo M**, Abreu-e-Lima P, Farraye F, Smith T, Swaroop P, Gardner L, Lauwers GY, Odze RD. Pathologic features of ulcerative colitis in patients with primary sclerosing cholangitis: a case-control study. *Am J Surg Pathol* 2009; **33**: 854-862

[PMID: 19295408 DOI: 10.1097/PAS.0b013e318196d018]

9 **Bergquist A**, Montgomery SM, Bahmanyar S, Olsson R, Danielsson A, Lindgren S, Prytz H, Hultcrantz R, Lööf LA, Sandberg-Gertzén H, Almer S, Askling J, Ehlin A, Ekbom A. Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 2008; **6**: 939-943 [PMID: 18674735 DOI: 10.1016/j.cgh.2008.03.016]

10 **Farrant JM**, Doherty DG, Donaldson PT, Vaughan RW, Hayllar KM, Welsh KI, Eddleston AL, Williams R. Amino acid substitutions at position 38 of the DR beta polypeptide confer susceptibility to and protection from primary sclerosing cholangitis. *Hepatology* 1992; **16**: 390-395 [PMID: 1639348 DOI: 10.1002/hep.1840160217]

11 **Olerup O**, Olsson R, Hultcrantz R, Broome U. HLA-DR and HLA-DQ are not markers for rapid disease progression in primary sclerosing cholangitis. *Gastroenterology* 1995; **108**: 870-878 [PMID: 7875491 DOI: 10.1016/0016-5085(95)90463-8]

12 **Janse M**, Lamberts LE, Franke L, Raychaudhuri S, Ellinghaus E, Muri Boberg K, Melum E, Folseraas T, Schrumpf E, Bergquist A, Björnsson E, Fu J, Jan Westra H, Groen HJ, Fehrmann RS, Smolonska J, van den Berg LH, Ophoff RA, Porte RJ, Weismüller TJ, Wedemeyer J, Schramm C, Sterneck M, Günther R, Braun F, Vermeire S, Henckaerts L, Wijmenga C, Ponsioen CY, Schreiber S, Karlsen TH, Franke A, Weersma RK. Three ulcerative colitis susceptibility loci are associated with primary sclerosing cholangitis and indicate a role for IL2, REL, and CARD9. *Hepatology* 2011; **53**: 1977-1985 [PMID: 21425313 DOI: 10.1002/hep.24307]

13 **Mulder AH**, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CG. Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver diseases. *Hepatology* 1993; **17**: 411-417 [PMID: 8444414 DOI: 10.1002/hep.1840170310]

14 **Bansi DS**, Fleming KA, Chapman RW. Importance of antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis and ulcerative colitis: prevalence, titre, and IgG subclass. *Gut* 1996; **38**: 384-389 [PMID: 8675091 DOI: 10.1136/gut.38.3.384]

15 **Terjung B**, Spengler U. Role of auto-antibodies for the diagnosis of chronic cholestatic liver diseases. *Clin Rev Allergy Immunol* 2005; **28**: 115-133 [PMID: 15879618 DOI: 10.1385/CRIAI:28:2:115]

16 **Angulo P**, Peter JB, Gershwin ME, DeSotel CK, Shoenfeld Y, Ahmed AE, Lindor KD. Serum autoantibodies in patients with primary sclerosing cholangitis. *J Hepatol* 2000; **32**: 182-187 [PMID: 10707856 DOI: 10.1016/S0168-8278(00)80061-6]

17 **Aoki CA**, Bowlus CL, Gershwin ME. The immunobiology of primary sclerosing cholangitis. *Autoimmun Rev* 2005; **4**: 137-143 [PMID: 15823499 DOI: 10.1016/j.autrev.2004.09.003]

18 **Loftus EV**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511 DOI: 10.1136/gut.2004.046615]

19 **Heuschen UA**, Hinz U, Allemeyer EH, Stern J, Lucas M, Autschbach F, Herfarth C, Heuschen G. Backwash ileitis is strongly associated with colorectal carcinoma in ulcerative colitis. *Gastroenterology* 2001; **120**: 841-847 [PMID: 11231938 DOI: 10.1053/gast.2001.22434]

20 **Penna C**, Dozois R, Tremaine W, Sandborn W, LaRusso N, Schleck C, Ilstrup D. Pouchitis after ileal pouch-anal anastomosis for ulcerative colitis occurs with increased frequency in patients with associated primary sclerosing cholangitis. *Gut* 1996; **38**: 234-239 [PMID: 8801203 DOI: 10.1136/gut.38.2.234]

21 **Soetikno RM**, Lin OS, Heidenreich PA, Young HS, Blackstone MO. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. *Gastrointest Endosc* 2002; **56**: 48-54 [PMID: 12085034 DOI: 10.1067/mge.2002.125367]

22 **Lundqvist K**, Broomé U. Differences in colonic disease activity in patients with ulcerative colitis with and without primary sclerosing cholangitis: a case control study. *Dis Colon Rectum* 1997; **40**: 451-456 [PMID: 9106695 DOI: 10.1007/BF02258391]

23 **Navaneethan U**, Venkatesh PG, Mukewar S, Lashner BA, Remzi FH, McCullough AJ, Kiran RP, Shen B, Fung JJ. Progressive primary sclerosing cholangitis requiring liver transplantation is associated with reduced need for colectomy in patients with ulcerative colitis. *Clin Gastroenterol Hepatol* 2012; **10**: 540-546 [PMID: 22245961 DOI: 10.1016/j.cgh.2012.01.006]

24 **Marelli L**, Xirouchakis E, Kalambokis G, Cholongitas E, Hamilton MI, Burroughs AK. Does the severity of primary sclerosing cholangitis influence the clinical course of associated ulcerative colitis? *Gut* 2011; **60**: 1224-1228 [PMID: 21402617 DOI: 10.1136/gut.2010.235408]

25 **Uko V**, Thangada S, Radhakrishnan K. Liver disorders in inflammatory bowel disease. *Gastroenterol Res Pract* 2012; **2012**: 642923 [PMID: 22474447 DOI: 10.1155/2012/642923]

26 **Said K**, Glaumann H, Bergquist A. Gallbladder disease in patients with primary sclerosing cholangitis. *J Hepatol* 2008; **48**: 598-605 [PMID: 18222013 DOI: 10.1016/j.jhep.2007.11.019]

27 **Moff SL**, Kamel IR, Eustace J, Lawler LP, Kantsevoy S, Kalloo AN, Thuluvath PJ. Diagnosis of primary sclerosing cholangitis: a blinded comparative study using magnetic resonance cholangiography and endoscopic retrograde cholangiography. *Gastrointest Endosc* 2006; **64**: 219-223 [PMID: 16860072 DOI: 10.1016/j.gie.2005.12.034]

28 **Chapman R**, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]

29 **Ludwig J**, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. *Hepatology* 1981; **1**: 632-640 [PMID: 7308996 DOI: 10.1002/hep.1840010612]

30 **Broomé U**, Olsson R, Lööf L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, Sandberg-Gertzén H, Wallerstedt S, Lindberg G. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996; **38**: 610-615 [PMID: 8707097 DOI: 10.1136/gut.38.4.610]

31 **Ngu JH**, Gearry RB, Wright AJ, Stedman CA. Inflammatory bowel disease is associated with poor outcomes of patients with primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 2011; **9**: 1092-107; quiz e135 [PMID: 21893134 DOI: 10.1016/j.cgh.2011.08.027]

32 **Navaneethan U**, Venkatesh PG, Lashner BA, Shen B, Kiran RP. The Impact of ulcerative colitis on the long-term outcome of patients with primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2012; Epub ahead of print [PMID: 22428605 DOI: 10.1111/j.1365-2036.2012.05063]

33 **Kim WR**, Therneau TM, Wiesner RH, Poterucha JJ, Benson JT, Malinchoc M, LaRusso NF, Lindor KD, Dickson ER. A revised natural history model for primary sclerosing cholangitis. *Mayo Clin Proc* 2000; **75**: 688-694 [PMID: 10907383]

34 **Kato T**, Komori A, Bae SK, Migita K, Ito M, Motoyoshi Y, Abiru S, Ishibashi H. Concurrent systemic AA amyloidosis can discriminate primary sclerosing cholangitis from IgG4-associated cholangitis. *World J Gastroenterol* 2012; **18**: 192-196 [PMID: 22253527 DOI: 10.3748/wjg.v18.i2.192]

35 **Bergquist A**, Ekbom A, Olsson R, Kornfeldt D, Lööf L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzén H, Almer S, Granath F, Broomé U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 2002; **36**: 321-327 [PMID: 11867174 DOI: 10.1016/S0168-8278(01)00288-4]

36 **Burak K**, Angulo P, Pasha TM, Egan K, Petz J, Lindor KD. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. *Am J Gastroenterol* 2004; **99**: 523-526 [PMID: 15056096 DOI: 10.1111/j.1572-0241.2004.04067.x]

37 **Boberg KM**, Jebsen P, Clausen OP, Foss A, Aabakken L, Schrumpf E. Diagnostic benefit of biliary brush cytology in cholangiocarcinoma in primary sclerosing cholangitis. *J Hepatol* 2006; **45**: 568-574 [PMID: 16879890 DOI: 10.1016/j.jhep.2006.05.010]

38 **Marsh JW**, Iwatsuki S, Makowka L, Esquivel CO, Gordon RD, Todo S, Tzakis A, Miller C, Van Thiel D, Starzl TE. Orthotopic liver transplantation for primary sclerosing cholangitis. *Ann Surg* 1988; **207**: 21-25 [PMID: 2827593 DOI: 10.1097/00000658-198801000-00005]

39 **Graziadei IW**, Wiesner RH, Batts KP, Marotta PJ, LaRusso NF, Porayko MK, Hay JE, Gores GJ, Charlton MR, Ludwig J, Poterucha JJ, Steers JL, Krom RA. Recurrence of primary sclerosing cholangitis following liver transplantation. *Hepatology* 1999; **29**: 1050-1056 [PMID: 10094945 DOI: 10.1002/hep.510290427]

40 **Bleday R**, Lee E, Jessurun J, Heine J, Wong WD. Increased risk of early colorectal neoplasms after hepatic transplant in patients with inflammatory bowel disease. *Dis Colon Rectum* 1993; **36**: 908-912 [PMID: 8404380 DOI: 10.1007/BF02050624]

41 **Navaneethan U**, Kochhar G, Venkatesh PG, Lewis B, Lashner BA, Remzi FH, Shen B, Kiran RP. Duration and severity of primary sclerosing cholangitis is not associated with risk of neoplastic changes in the colon in patients with ulcerative colitis. *Gastrointest Endosc* 2012; **75**: 1045-1054.e1 [PMID: 22405258 DOI: 10.1016/j.gie.2012.01.015]

42 **Ståhlberg D**, Veress B, Tribukait B, Broomé U. Atrophy and neoplastic transformation of the ileal pouch mucosa in patients with ulcerative colitis and primary sclerosing cholangitis: a case control study. *Dis Colon Rectum* 2003; **46**: 770-778 [PMID: 12794579]

43 **Chaparro M**, Trapero-Marugán M, Guijarro M, López C, Moreno-Otero R, Gisbert JP. Dysplasia and colorectal cancer in a patient with ulcerative colitis and primary sclerosing cholangitis: a case report and a short review of the literature. *J Crohns Colitis* 2013; **7**: e61-e65 [PMID: 22552273 DOI: 10.1016/j.crohns.2012.04.005]

44 **Lindor KD**, Kowdley KV, Luketic VA, Harrison ME, McCashland T, Befeler AS, Harnois D, Jorgensen R, Petz J, Keach J, Mooney J, Sargeant C, Braaten J, Bernard T, King D, Miceli E, Schmoll J, Hoskin T, Thapa P, Enders F. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 2009; **50**: 808-814 [PMID: 19585548 DOI: 10.1002/hep.23082]

45 **Shi J**, Li Z, Zeng X, Lin Y, Xie WF. Ursodeoxycholic acid in primary sclerosing cholangitis: meta-analysis of randomized controlled trials. *Hepatol Res* 2009; **39**: 865-873 [PMID: 19467021 DOI: 10.1111/j.1872-034X.2009.00527.x]

46 **Ashraf I**, Choudhary A, Arif M, Matteson ML, Hammad HT, Puli SR, Bechtold ML. Ursodeoxycholic acid in patients with ulcerative colitis and primary sclerosing cholangitis for prevention of colon cancer: a meta-analysis. *Indian J Gastroenterol* 2012; **31**: 69-74 [PMID: 22528343 DOI: 10.1007/s12664-012-0175-3]

47 **Graziadei IW**, Wiesner RH, Marotta PJ, Porayko MK, Hay JE, Charlton MR, Poterucha JJ, Rosen CB, Gores GJ, LaRusso NF, Krom RA. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. *Hepatology* 1999; **30**: 1121-1127 [PMID: 10534330]

48 **Baluyut AR**, Sherman S, Lehman GA, Hoen H, Chalasani N. Impact of endoscopic therapy on the survival of patients with primary sclerosing cholangitis. *Gastrointest Endosc* 2001; **53**: 308-312 [PMID: 11231388]

49 **Angulo P**, Maor-Kendler Y, Lindor KD. Small-duct primary sclerosing cholangitis: a long-term follow-up study. *Hepatology* 2002; **35**: 1494-1500 [PMID: 12029635 DOI: 10.1053/jhep.2002.33202]

50 **Björnsson E**, Olsson R, Bergquist A, Lindgren S, Braden B, Chapman RW, Boberg KM, Angulo P. The natural history of small-duct primary sclerosing cholangitis. *Gastroenterology* 2008; **134**: 975-980 [PMID: 18395078 DOI: 10.1053/j.gastro.2008.01.042]

51 **Woodward J**, Neuberger J. Autoimmune overlap syndromes. *Hepatology* 2001; **33**: 994-1002 [PMID: 11283866 DOI: 10.1053/jhep.2001.23316]

52 **Gregorio GV**, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, Mieli-Vergani G. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. *Hepatology* 2001; **33**: 544-553 [PMID: 11230733 DOI: 10.1053/jhep.2001.22131]

53 **Czaja AJ**. Frequency and nature of the variant syndromes of autoimmune liver disease. *Hepatology* 1998; **28**: 360-365 [PMID: 9695997 DOI: 10.1002/hep.510280210]

54 **Lüth S**, Kanzler S, Frenzel C, Kasper HU, Dienes HP, Schramm C, Galle PR, Herkel J, Lohse AW. Characteristics and long-term prognosis of the autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. *J Clin Gastroenterol* 2009; **43**: 75-80 [PMID: 18769363 DOI: 10.1097/MCG.0b013e318157c614]

55 **Santos OM**, Muñoz Ortiz E, Pérez C, Restrepo JC. [Autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome in adults: report of three cases]. *Gastroenterol Hepatol* 2012; **35**: 254-258 [PMID: 22284044 DOI: 10.1016/j.gastrohep.2011.12.003]

56 **Malik TA**, Gutierrez AM, McGuire B, Zarzour JG, Mukhtar F, Bloomer J. Autoimmune hepatitis-primary sclerosing cholangitis overlap syndrome complicated by Crohn's disease. *Digestion* 2010; **82**: 24-26 [PMID: 20160443 DOI: 10.1159/000273735]

57 **Czaja AJ**. The overlap syndromes of autoimmune hepatitis. *Dig Dis Sci* 2013; **58**: 326-343 [PMID: 22918690 DOI: 10.1007/s10620-012-2367-1]

58 **Dastis SN**, Latinne D, Sempoux C, Geubel AP. Ulcerative colitis associated with IgG4 cholangitis: similar features in two HLA identical siblings. *J Hepatol* 2009; **51**: 601-605 [PMID: 19615774 DOI: 10.1016/j.jhep.2009.05.032]

59 **Okazaki K**, Uchida K, Koyabu M, Miyoshi H, Takaoka M. Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease. *J Gastroenterol* 2011; **46**: 277-288 [PMID: 21452084 DOI: 10.1007/s00535-011-0386-x]

60 **Hirano K**, Kawabe T, Yamamoto N, Nakai Y, Sasahira N, Tsujino T, Toda N, Isayama H, Tada M, Omata M. Serum IgG4 concentrations in pancreatic and biliary diseases. *Clin Chim Acta* 2006; **367**: 181-184 [PMID: 16426597 DOI: 10.1016/j.cca.2005.11.031]

61 **Mendes FD**, Jorgensen R, Keach J, Katzmann JA, Smyrk T, Donlinger J, Chari S, Lindor KD. Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. *Am J Gastroenterol* 2006; **101**: 2070-2075 [PMID: 16879434 DOI: 10.1111/j.1572-0241.2006.00772.x]

62 **Novotný I**, Dítě P, Trna J, Lata J, Husová L, Geryk E. Immunoglobulin G4-related cholangitis: a variant of IgG4-related systemic disease. *Dig Dis* 2012; **30**: 216-219 [PMID: 22722442 DOI: 10.1159/000336706]

63 **Culver EL**, Chapman RW. Systematic review: management options for primary sclerosing cholangitis and its variant forms - IgG4-associated cholangitis and overlap with autoimmune hepatitis. *Aliment Pharmacol Ther* 2011; **33**: 1273-1291 [PMID: 21501198 DOI: 10.1111/j.1365-2036.2011.04658.x]

64 **Silveira MG**. IgG4-associated cholangitis. *Clin Liver Dis* 2013; **17**: 255-268 [PMID: 23540501 DOI: 10.1016/j.cld.2012.11.007]

65 **Tada F**, Abe M, Nunoi H, Azemoto N, Mashiba T, Furukawa S, Kumagi T, Murakami H, Ikeda Y, Matsuura B, Hiasa Y, Onji M. Ulcerative colitis complicated with primary biliary cirrhosis. *Intern Med* 2011; **50**: 2323-2327 [PMID: 22001458

DOI: 10.2169/internalmedicine.50.5919]

66 **Xiao WB**, Liu YL. Primary biliary cirrhosis and ulcerative colitis: a case report and review of literature. *World J Gastroenterol* 2003; **9**: 878-880 [PMID: 12679954]

67 **Ohge H**, Takesue Y, Yokoyama T, Hiyama E, Murakami Y, Imamura Y, Shimamoto F, Matsuura Y. Progression of primary biliary cirrhosis after proctocolectomy for ulcerative colitis. *J Gastroenterol* 2000; **35**: 870-872 [PMID: 11085498 DOI: 10.1007/s005350070026]

68 **Greenstein AJ**, Sachar DB, Panday AK, Dikman SH, Meyers S, Heimann T, Gumaste V, Werther JL, Janowitz HD. Amyloidosis and inflammatory bowel disease. A 50-year experience with 25 patients. *Medicine (Baltimore)* 1992; **71**: 261-270 [PMID: 1522802 DOI: 10.1097/00005792-199209000-00001]

69 **Wester AL**, Vatn MH, Fausa O. Secondary amyloidosis in inflammatory bowel disease: a study of 18 patients admitted to Rikshospitalet University Hospital, Oslo, from 1962 to 1998. *Inflamm Bowel Dis* 2001; **7**: 295-300 [PMID: 11720318 DOI: 10.1097/00054725-200111000-00003]

70 **Braun M**, Fraser GM, Kunin M, Salamon F, Tur-Kaspa R. Mesalamine-induced granulomatous hepatitis. *Am J Gastroenterol* 1999; **94**: 1973-1974 [PMID: 10406274 DOI: 10.1111/j.1572-0241.1999.01245.x]

71 **Venkatesh PG**, Navaneethan U, Shen B. Hepatobiliary disorders and complications of inflammatory bowel disease. *J Dig Dis* 2011; **12**: 245-256 [PMID: 21791019 DOI: 10.1111/j.1751-2980.2011.00511.x]

72 **Parente F**, Pastore L, Bargiggia S, Cucino C, Greco S, Molteni M, Ardizzone S, Porro GB, Sampietro GM, Giorgi R, Moretti R, Gallus S. Incidence and risk factors for gallstones in patients with inflammatory bowel disease: a large case-control study. *Hepatology* 2007; **45**: 1267-1274 [PMID: 17464998 DOI: 10.1002/hep.21537]

73 **Navaneethan U**, Shen B. Hepatopancreatobiliary manifestations and complications associated with inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 1598-1619 [PMID: 20198712 DOI: 10.1002/ibd.21219]

74 **Mibu R**, Makino I, Chijiiwa K. Gallstones and their composition in patients with ileoanal anastomosis. *J Gastroenterol* 1995; **30**: 413-415 [PMID: 7647911 DOI: 10.1007/BF02347521]

75 **Talbot RW**, Heppell J, Dozois RR, Beart RW. Vascular complications of inflammatory bowel disease. *Mayo Clin Proc* 1986; **61**: 140-145 [PMID: 3080643 DOI: 10.1016/S0025-6196(12)65200-8]

76 **Sridhar AR**, Parasa S, Navaneethan U, Crowell MD, Olden K. Comprehensive study of cardiovascular morbidity in hospitalized inflammatory bowel disease patients. *J Crohns Colitis* 2011; **5**: 287-294 [PMID: 21683298 DOI: 10.1016/j.crohns.2011.01.011]

77 **Danese S**, Papa A, Saibeni S, Repici A, Malesci A, Vecchi M. Inflammation and coagulation in inflammatory bowel disease: The clot thickens. *Am J Gastroenterol* 2007; **102**: 174-186 [PMID: 17100967 DOI: 10.1111/j.1572-0241.2006.00943.x]

78 **Solem CA**, Loftus EV, Tremaine WJ, Sandborn WJ. Venous thromboembolism in inflammatory bowel disease. *Am J Gastroenterol* 2004; **99**: 97-101 [PMID: 14687149 DOI: 10.1046/j.1572-0241.2003.04026.x]

79 **Sinagra E**, Aragona E, Romano C, Maisano S, Orlando A, Virdone R, Tesè L, Modesto I, Criscuoli V, Cottone M. The role of portal vein thrombosis in the clinical course of inflammatory bowel diseases: report on three cases and review of the literature. *Gastroenterol Res Pract* 2012; **2012**: 916428 [PMID: 23093957 DOI: 10.1155/2012/916428]

80 **Molina Infante J**, Bañares Cañizares R, Gómez Camarero J, Pérez Calle JL. [Liver abscess and Crohn' disease. Report of 3 cases]. *Gastroenterol Hepatol* 2004; **27**: 317-319 [PMID: 15117612]

81 **Bernabeu JL**, Leo E, Trigo C, Herrera JM, Sousa JM, Marquez JL. Crohn's disease and liver abscess due to Pediococcus sp. *Inflamm Bowel Dis* 2011; **17**: 2207-2208 [PMID: 21287670 DOI: 10.1002/ibd.21622]

82 **Aguas M**, Bastida G, Nos P, Beltrán B, Grueso JL, Grueso J. Septic thrombophlebitis of the superior mesenteric vein and multiple liver abscesses in a patient with Crohn's disease at onset. *BMC Gastroenterol* 2007; **7**: 22 [PMID: 17565671 DOI: 10.1186/1471-230X-7-22]

83 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]

84 **McGowan CE**, Jones P, Long MD, Barritt AS. Changing shape of disease: nonalcoholic fatty liver disease in Crohn' s disease-a case series and review of the literature. *Inflamm Bowel Dis* 2012; **18**: 49-54 [PMID: 21351214 DOI: 10.1002/ibd.21669]

85 **Sourianarayanane A**, Garg G, Smith TH, Butt MI, McCullough AJ, Shen B. Risk factors of non-alcoholic fatty liver disease in patients with inflammatory bowel disease. *J Crohns Colitis* 2013; **7**: e279-e285 [PMID: 23158500 DOI: 10.1016/j.crohns.2012.10.015]

86 **World Health Organization**. Immunization, vaccines and biologicals, hepatitis B. Available from: URL: http://www.who.int/immunization/topics/hepatitis_b/en/index.html Accessed November 11, 2008

87 NIH Consensus Statement on Management of Hepatitis C: 2002. *NIH Consens State Sci Statements* 2002; **19**: 1-46 [PMID: 14768714]

88 **Buti M**, García-Samaniego J, Prieto M, Rodríguez M, Sánchez-Tapias JM, Suárez E, Esteban R. [Consensus document of the Spanish Association for the Study of the Liver on the treatment of hepatitis B infection (2012)]. *Gastroenterol Hepatol* 2012; **35**: 512-528 [PMID: 22749508 DOI: 10.1016/j.gastrohep.2012.04.006]

89 **Katz LH**, Fraser A, Gafter-Gvili A, Leibovici L, Tur-Kaspa R. Lamivudine prevents reactivation of hepatitis B and reduces mortality in immunosuppressed patients: systematic review and meta-analysis. *J Viral Hepat* 2008; **15**: 89-102 [PMID: 18184191 DOI: 10.1111/j.1365-2893.2007.00902.x]

90 **Hui CK**, Cheung WW, Zhang HY, Au WY, Yueng YH, Leung AY, Leung N, Luk JM, Lie AK, Kwong YL, Liang R, Lau GK. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; **131**: 59-68 [PMID: 16831590 DOI: 10.1053/j.gastro.2006.04.015]

91 **Li HR**, Huang JJ, Guo HQ, Zhang X, Xie Y, Zhu HL, Zhai LZ, Pu XX, Huang Y, Guo CC, Lin TY. Comparison of entecavir and lamivudine in preventing hepatitis B reactivation in lymphoma patients during chemotherapy. *J Viral Hepat* 2011; **18**: 877-883 [PMID: 21054683 DOI: 10.1111/j.1365-2893.2010.01386.x]

92 **Mahale P**, Kontoyiannis DP, Chemaly RF, Jiang Y, Hwang JP, Davila M, Torres HA. Acute exacerbation and reactivation of chronic hepatitis C virus infection in cancer patients. *J Hepatol* 2012; **57**: 1177-1185 [PMID: 22871500 DOI: 10.1016/j.jhep.2012.07.031]

93 **Biancone L**, Pavia M, Del Vecchio Blanco G, D'Incà R, Castiglione F, De Nigris F, Doldo P, Cosco F, Vavassori P, Bresci GP, Arrigoni A, Cadau G, Monteleone I, Rispo A, Fries W, Mallardi B, Sturniolo GC, Pallone F. Hepatitis B and C virus infection in Crohn's disease. *Inflamm Bowel Dis* 2001; **7**: 287-294 [PMID: 11720317]

94 **Esteve M**, Saro C, González-Huix F, Suarez F, Forné M, Viver JM. Chronic hepatitis B reactivation following infliximab therapy in Crohn's disease patients: need for primary prophylaxis. *Gut* 2004; **53**: 1363-1365 [PMID: 15306601 DOI: 10.1136/gut.2004.040675]

95 **Loras C**, Saro C, Gonzalez-Huix F, Mínguez M, Merino O, Gisbert JP, Barrio J, Bernal A, Gutiérrez A, Piqueras M, Calvet X, Andreu M, Abad A, Ginard D, Bujanda L, Panés J, Torres M, Fernández-Bañares F, Viver JM, Esteve M. Preva-

lence and factors related to hepatitis B and C in inflammatory bowel disease patients in Spain: a nationwide, multicenter study. *Am J Gastroenterol* 2009; **104**: 57-63 [PMID: 19098850 DOI: 10.1038/ajg.2008.4]

96 **Chevaux JB**, Bigard MA, Bensenane M, Oussalah A, Jarlot S, Belle A, Nani A, Bronowicki JP, Peyrin-Biroulet L. Inflammatory bowel disease and hepatitis B and C. *Gastroenterol Clin Biol* 2009; **33**: 1082-1093 [PMID: 19896313 DOI: 10.1016/j.gcb.2009.03.021]

97 **Zeitz J**, Mullhaupt B, Fruehauf H, Rogler G, Vavricka SR. Hepatic failure due to hepatitis B reactivation in a patient with ulcerative colitis treated with prednisone. *Hepatology* 2009; **50**: 653-654 [PMID: 19575458 DOI: 10.1002/hep.23035]

98 **Kim YJ**, Lee HS, Yoon JH, Kim CY, Park MH, Kim LH, Park BL, Shin HD. Association of TNF-alpha promoter polymorphisms with the clearance of hepatitis B virus infection. *Hum Mol Genet* 2003; **12**: 2541-2546 [PMID: 12915457]

99 **Millonig G**, Kern M, Ludwiczek O, Nachbaur K, Vogel W. Subfulminant hepatitis B after infliximab in Crohn's disease: need for HBV-screening? *World J Gastroenterol* 2006; **12**: 974-976 [PMID: 16521231 DOI: 10.1093/hmg/ddg262]

100 **Colbert C**, Chavarria A, Berkelhammer C. Fulminant hepatic failure in chronic hepatitis B on withdrawal of corticosteroids, azathioprine and infliximab for Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 1453-1454 [PMID: 17600380 DOI: 10.1002/ibd.20216]

101 **Ojiro K**, Naganuma M, Ebinuma H, Kunimoto H, Tada S, Ogata H, Iwao Y, Saito H, Hibi T. Reactivation of hepatitis B in a patient with Crohn's disease treated using infliximab. *J Gastroenterol* 2008; **43**: 397-401 [PMID: 18592158 DOI: 10.1007/s00535-008-2165-x]

102 **Ueno Y**, Tanaka S, Shimamoto M, Miyanaka Y, Hiyama T, Ito M, Kitadai Y, Yoshihara M, Sumii M, Chayama K. Infliximab therapy for Crohn's disease in a patient with chronic hepatitis B. *Dig Dis Sci* 2005; **50**: 163-166 [PMID: 15712655 DOI: 10.1007/s10620-005-1295-8]

103 **Madonia S**, Orlando A, Scimeca D, Olivo M, Rossi F, Cottone M. Occult hepatitis B and infliximab-induced HBV reactivation. *Inflamm Bowel Dis* 2007; **13**: 508-509 [PMID: 17206687 DOI: 10.1002/ibd.20035]

104 **Loras C**, Gisbert JP, Mínguez M, Merino O, Bujanda L, Saro C, Domenech E, Barrio J, Andreu M, Ordás I, Vida L, Bastida G, González-Huix F, Piqueras M, Ginard D, Calvet X, Gutiérrez A, Abad A, Torres M, Panés J, Chaparro M, Pascual I, Rodriguez-Carballeira M, Fernández-Bañares F, Viver JM, Esteve M. Liver dysfunction related to hepatitis B and C in patients with inflammatory bowel disease treated with immunosuppressive therapy. *Gut* 2010; **59**: 1340-1346 [PMID: 20577000 DOI: 10.1136/gut.2010.208413]

105 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]

106 **Rahier JF**, Ben-Horin S, Chowers Y, Conlon C, De Munter P, D'Haens G, Domènech E, Eliakim R, Eser A, Frater J, Gassull M, Giladi M, Kaser A, Lémann M, Moreels T, Moschen A, Pollok R, Reinisch W, Schunter M, Stange EF, Tilg H, Van Assche G, Viget N, Vucelic B, Walsh A, Weiss G, Yazdanpanah Y, Zabana Y, Travis SP, Colombel JF. European evidence-based Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2009; **3**: 47-91 [PMID: 21172250 DOI: 10.1016/j.crohns.2009.02.010]

107 **Gisbert JP**, Villagrasa JR, Rodríguez-Nogueiras A, Chaparro M. Efficacy of hepatitis B vaccination and revaccination and factors impacting on response in patients with inflammatory bowel disease. *Am J Gastroenterol* 2012; **107**: 1460-1466 [PMID: 23034605 DOI: 10.1038/ajg.2012.79]

108 **Biancone L**, Del Vecchio Blanco G, Pallone F, Castiglione F, Bresci G, Sturniolo G. Immunomodulatory drugs in Crohn's disease patients with hepatitis B or C virus infection. *Gastroenterology* 2002; **122**: 593-594 [PMID: 11845808 DOI: 10.1053/gast.2002.31602]

109 **Schiavon LL**, Carvalho-Filho RJ, Narciso-Schiavon JL, Barbosa DV, Lanzoni VP, Ferraz ML, Silva AE. Impact of cyclosporine-based immunosuppressive therapy on liver histology of hepatitis C virus-infected renal transplant patients. *Hepatology* 2008; **48**: 348-349 [PMID: 18521870 DOI: 10.1002/hep.22331]

110 **Samonakis DN**, Triantos CK, Thalheimer U, Quaglia A, Leandro G, Teixeira R, Papatheodoridis GV, Sabin CA, Rolando N, Davies S, Dhillon AP, Griffiths P, Emery V, Patch DW, Davidson BR, Rolles K, Burroughs AK. Immunosuppression and donor age with respect to severity of HCV recurrence after liver transplantation. *Liver Transpl* 2005; **11**: 386-395 [PMID: 15776454 DOI: 10.1002/lt.20344]

111 **Zekry A**, Gleeson M, Guney S, McCaughan GW. A prospective cross-over study comparing the effect of mycophenolate versus azathioprine on allograft function and viral load in liver transplant recipients with recurrent chronic HCV infection. *Liver Transpl* 2004; **10**: 52-57 [PMID: 14755778 DOI: 10.1002/lt.20000]

112 **Nissen MJ**, Fontanges E, Allam Y, Zoulim F, Trépo C, Miossec P. Rheumatological manifestations of hepatitis C: incidence in a rheumatology and non-rheumatology setting and the effect of methotrexate and interferon. *Rheumatology (Oxford)* 2005; **44**: 1016-1020 [PMID: 15855185 DOI: 10.1093/rheumatology/keh668]

113 **Fukuda R**, Ishimura N, Ishihara S, Chowdhury A, Morlyama N, Nogami C, Miyake T, Niigaki M, Tokuda A, Satoh S, Sakai S, Akagi S, Watanabe M, Fukumoto S. Intrahepatic expression of pro-inflammatory cytokine mRNAs and interferon efficacy in chronic hepatitis C. *Liver* 1996; **16**: 390-399 [PMID: 9021719 DOI: 10.1111/j.1600-0676.1996.tb00768.x]

114 **Zein NN**. Etanercept as an adjuvant to interferon and ribavirin in treatment-naive patients with chronic hepatitis C virus infection: a phase 2 randomized, double-blind, placebo-controlled study. *J Hepatol* 2005; **42**: 315-322 [PMID: 15791697 DOI: 10.1016/j.jhep.2004.11.025]

115 **Peterson JR**, Hsu FC, Simkin PA, Wener MH. Effect of tumour necrosis factor alpha antagonists on serum transaminases and viraemia in patients with rheumatoid arthritis and chronic hepatitis C infection. *Ann Rheum Dis* 2003; **62**: 1078-1082 [PMID: 14583571 DOI: 10.1136/ard.62.11.1078]

116 **Villa F**, Rumi MG, Signorelli C, Saibeni S, Del Ninno E, Ferrero Bogetto S, de Franchis R, Vecchi M. Onset of inflammatory bowel diseases during combined alpha-interferon and ribavirin therapy for chronic hepatitis C: report of two cases. *Eur J Gastroenterol Hepatol* 2005; **17**: 1243-1245 [PMID: 16215439 DOI: 10.1097/00042737-200511000-00015]

117 **Abdelmalek MF**, Liu C, Valentine JF. Successful treatment of chronic hepatitis C with pegylated interferon, ribavirin, and infliximab in a patient with Crohn's disease. *Am J Gastroenterol* 2007; **102**: 1333-1334 [PMID: 17531027 DOI: 10.1111/j.1572-0241.2007.01131.x]

118 **Salcedo-Mora X**, Maté J, Medina J, Nam Cha SJ, Gisbert JP, Moreno-Otero R. Chronic hepatitis C and Crohn's disease: nosocomial infection treatment with PEG-interferon plus ribavirin. *Digestion* 2006; **73**: 210-214 [PMID: 16883071 DOI: 10.1159/000094787]

119 **Scherzer TM**, Staufer K, Novacek G, Steindl-Munda P, Schumacher S, Hofer H, Ferenci P, Vogelsang H. Efficacy and safety of antiviral therapy in patients with Crohn's disease and chronic hepatitis C. *Aliment Pharmacol Ther* 2008; **28**: 742-748 [PMID: 19145730 DOI: 10.1111/j.1365-2036.2008.03779.x]

120 **Sümer N**, Palabiyikoğlu M. Induction of remission by interferon-alpha in patients with chronic active ulcerative colitis. *Eur J Gastroenterol Hepatol* 1995; **7**: 597-602 [PMID: 8590152]

121 **Musch E**, Andus T, Malek M. Induction and maintenance of

clinical remission by interferon-beta in patients with steroid-refractory active ulcerative colitis-an open long-term pilot trial. *Aliment Pharmacol Ther* 2002; **16**: 1233-1239 [PMID: 12144572 DOI: 10.1046/j.1365-2036.2002.01264.x]
122 **Seow CH**, Benchimol EI, Griffiths AM, Steinhart AH. Type I interferons for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2008; (**3**): CD006790 [PMID: 18646167 DOI: 10.1002/14651858.CD006790.pub2]
123 **De Diego Lorenzo A**, Kashoob M, Romero M, Parera A, Durán F, Piqueras B, Santos L, Cos E, Clemente G. [Treatment with recombinant interferon alfa-2b of a patient with chronic hepatitis C and concomitant ulcerative colitis]. *Rev Esp Enferm Dig* 1997; **89**: 399-401 [PMID: 9280430]
124 **Mitoro A**, Yoshikawa M, Yamamoto K, Mimura M, Yoshikawa Y, Shiroi A, Mochi T, Sakamoto T, Yamao J, Kikuchi E. Exacerbation of ulcerative colitis during alpha-interferon therapy for chronic hepatitis C. *Intern Med* 1993; **32**: 327-331 [PMID: 8102914 DOI: 10.2169/internalmedicine.32.327]
125 **Bargiggia S**, Thorburn D, Anderloni A, Ardizzone S, Giorgi A, Bianchi Porro G, Parente F. Is interferon-alpha therapy safe and effective for patients with chronic hepatitis C and inflammatory bowel disease? A case-control study. *Aliment Pharmacol Ther* 2005; **22**: 209-215 [PMID: 16091058 DOI: 10.1111/j.1365-2036.2005.02556.x]
126 **Horn TL**, Reynolds J, de Villiers W, Peña LR. Hepatitis C virus and inflammatory bowel disease. *Dig Dis Sci* 2009; **54**: 1171-1177 [PMID: 18975088 DOI: 10.1007/s10620-008-0489-2]
127 **Gisbert JP**, Luna M, González-Lama Y, Pousa ID, Velasco M, Moreno-Otero R, Maté J. Liver injury in inflammatory bowel disease: long-term follow-up study of 786 patients. *Inflamm Bowel Dis* 2007; **13**: 1106-1114 [PMID: 17455203 DOI: 10.1002/ibd.20160]
128 **Loftus EV**, Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2004; **19**: 179-189 [PMID: 14723609 DOI: 10.1111/j.0269-2813.2004.01827.x]
129 **Ransford RA**, Langman MJ. Sulphasalazine and mesalazine: serious adverse reactions re-evaluated on the basis of suspected adverse reaction reports to the Committee on Safety of Medicines. *Gut* 2002; **51**: 536-539 [PMID: 12235076 DOI: 10.1136/gut.51.4.536]
130 **Saibeni S**, Bollani S, Losco A, Michielan A, Sostegni R, Devani M, Lupinacci G, Pirola L, Cucino C, Meucci G, Basilisco G, D'Incà R, Bruno S. The use of methotrexate for treatment of inflammatory bowel disease in clinical practice. *Dig Liver Dis* 2012; **44**: 123-127 [PMID: 22051323 DOI: 10.1016/j.dld.2011.09.015]
131 **Te HS**, Schiano TD, Kuan SF, Hanauer SB, Conjeevaram HS, Baker AL. Hepatic effects of long-term methotrexate use in the treatment of inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 3150-3156 [PMID: 11095334 DOI: 10.1111/j.1572-0241.2000.03287.x]
132 **Chande N**, Ponich T, Gregor J. A survey of Canadian gastroenterologists about the use of methotrexate in patients with Crohn's disease. *Can J Gastroenterol* 2005; **19**: 553-558 [PMID: 16151547]
133 **Chande N**, Abdelgadir I, Gregor J. The safety and tolerability of methotrexate for treating patients with Crohn's disease. *J Clin Gastroenterol* 2011; **45**: 599-601 [PMID: 21224737 DOI: 10.1097/MCG.0b013e3181f593f9]
134 **Barbero-Villares A**, Mendoza J, Trapero-Marugan M, Gonzalez-Alvaro I, Daudén E, Gisbert JP, Moreno-Otero R. Evaluation of liver fibrosis by transient elastography in methotrexate treated patients. *Med Clin (Barc)* 2011; **137**: 637-639 [PMID: 21719043 DOI: 10.1016/j.medcli.2010.12.024]
135 **Dominique L**. Liver toxicity of TNFalpha antagonists. *Joint Bone Spine* 2008; **75**: 636-638 [PMID: 18952478 DOI: 10.1016/j.jbspin.2008.07.005]
136 FDA Briefing Document. Arthritis Advisory Committee (15 Jun 2009). Available from: URL: http://ww.fda.gov/ohrms/dockets/act/03/transcripts/3930T1.htm
137 **Ghabril M**, Bonkovsky HL, Kum C, Davern T, Hayashi PH, Kleiner DE, Serrano J, Rochon J, Fontana RJ, Bonacini M. Liver injury from tumor necrosis factor-α antagonists: analysis of thirty-four cases. *Clin Gastroenterol Hepatol* 2013; **11**: 558-564.e3 [PMID: 23333219 DOI: 10.1016/j.cgh.2012.12.025]
138 **Titos Arcos JC**, Hallal H, Robles M, Andrade RJ. Recurrent hepatotoxicity associated with etanercept and adalimumab but not with infliximab in a patient with rheumatoid arthritis. *Rev Esp Enferm Dig* 2012; **104**: 282-284 [PMID: 22662786 DOI: 10.4321/S1130-01082012000500014]
139 **Bastida G**, Nos P, Aguas M, Beltrán B, Rubín A, Dasí F, Ponce J. Incidence, risk factors and clinical course of thiopurine-induced liver injury in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2005; **22**: 775-782 [PMID: 16225485 DOI: 10.1111/j.1365-2036.2005.02636.x]
140 **Russmann S**, Zimmermann A, Krähenbühl S, Kern B, Reichen J. Veno-occlusive disease, nodular regenerative hyperplasia and hepatocellular carcinoma after azathioprine treatment in a patient with ulcerative colitis. *Eur J Gastroenterol Hepatol* 2001; **13**: 287-290 [PMID: 11293451 DOI: 10.1097/00042737-200103000-00013]
141 **Haboubi NY**, Ali HH, Whitwell HL, Ackrill P. Role of endothelial cell injury in the spectrum of azathioprine-induced liver disease after renal transplant: light microscopy and ultrastructural observations. *Am J Gastroenterol* 1988; **83**: 256-261 [PMID: 3278593]
142 **Gisbert JP**, González-Lama Y, Maté J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. *Am J Gastroenterol* 2007; **102**: 1518-1527 [PMID: 17391318 DOI: 10.1111/j.1572-0241.2007.01187.x]

P- Reviewers: Ingle SB, Thorne K **S- Editor**: Song XX
L- Editor: Rutherford A **E- Editor**: Zhang DN

WJG
World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7341

World J Gastroenterol 2013 November 14; 19(42): 7341-7360
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

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REVIEW

Physiological and molecular biochemical mechanisms of bile formation

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Received: June 28, 2013 **Revised:** September 27, 2013
Accepted: September 29, 2013
Published online: November 14, 2013

Abstract

This review considers the physiological and molecular biochemical mechanisms of bile formation. The composition of bile and structure of a bile canaliculus, biosynthesis and conjugation of bile acids, bile phospholipids, formation of bile micellar structures, and enterohepatic circulation of bile acids are described. In general, the review focuses on the molecular physiology of the transporting systems of the hepatocyte sinusoidal and apical membranes. Knowledge of physiological and biochemical basis of bile formation has implications for understanding the mechanisms of development of pathological processes, associated with diseases of the liver and biliary tract.

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Key words: Bile acids; Bile phospholipids; Bile micelle structures; Bile salt transporters

Core tip: Over the past 50 years, significant progress has been made in understanding the mechanisms of bile formation and secretion. This became possible due to the advances of fundamental investigations in cell biology, molecular biology, genetics and biochemistry. Knowledge of physiological and biochemical basis of bile formation has implications for understanding the mechanisms of development of pathological processes, associated with diseases of the liver and biliary tract.

Reshetnyak VI. Physiological and molecular biochemical mechanisms of bile formation. *World J Gastroenterol* 2013; 19(42): 7341-7360 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7341.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7341

INTRODUCTION

Bile is a lipid-rich hepatic secretion that is necessary for elimination of cholesterol and xenobiotics from the body and for dispersion and efficient absorption of digested dietary lipid in the upper small intestine[1]. Biliary function is a vital function of the liver, which results from the sequential vectorial transport of endogenous and exogenous substrates through three compartments: the vascular space, cellular space and biliary space. Canalicular bile is produced by polarized hepatocytes that hold transporters in their basolateral (sinusoidal) and apical (canalicular) plasma membrane[2]. The biliary function is responsible for the homeostasis of lipid metabolism, in particular cholesterol metabolism, elimination of toxic endo- and xenobiotics such as bilirubin, lipid bacteria products (endotoxin), and several inflammatory mediators[3]. Bile coming into the canaliculi is modified by cholangiocytes through secretion and absorption. The main determinant of bile formation is an osmotic filtration process resulting from active transport of bile acids and other osmotic solutes[3]. Pavlov *et al*[4] established the basic mechanisms of bile secretion, its entry into the duodenum, and the role of bile in digestion. Bile is essential for the intestinal digestion and absorption of nutriments. Almost half a century had passed before the experimentally founded ideas on the mechanisms of bile formation appeared. Later, they were clarified and in the late 1970s took shape as a number of hypotheses of the mechanisms of bile formation. Recent insights into the cellular and molecular mechanisms that control the function and regulation of hepatobiliary transport have led to a greater understanding of the physiological significance of bile secretion. Individual carriers for bile acids and other organic anions in the liver and intestine have now been obtained from several species. In addition, complex networks of signals that regulate key enzymes and membrane transporters located in cells that participate in the metabolism or transport of biliary constituents are being unraveled[5]. Most of the membrane transporters ensuring bile formation have now been identified. The expression of these membrane transporters is regulated through transcriptional and post-transductional mechanisms. Transcriptional regulation is under the control of nuclear receptors activated by ligands such as bile acids, which act as endogenous steroids synthesized from cholesterol in hepatocytes[3].

Bile is an iso-osmotic electrolytic fluid that is formed in the liver and is a product of its secretory function. Bile is primarily secreted by hepatocytes (*i.e.*, canalicular bile) and subsequently delivered to the intrahepatic bile ducts, where it is modified by cholangiocytes (*i.e.*, ductal bile). Bile secretion by liver parenchymal cells is the result of vectorial transcellular transport of solutes and involves the coordinated action of transport proteins at the basolateral (sinusoidal) and apical (canalicular) membranes of the hepatocyte. A complex network of signals controls uptake and efflux transporters on a long- and short-term timescale, including regulation at the level of gene transcription, protein translation and maturation, covalent modification, and dynamic localization of transporter proteins, as well as substrate availability[6].

COMPOSITION OF BILE AND STRUCTURE OF A BILE CANALICULUS

Bile contains almost all body components: proteins, lipids, carbohydrates, vitamins, mineral salts, and trace elements. The greater part of the bile proteins consists of globulins, and the lesser part comprises albumins. Phospholipids, cholesterol and its esters, neutral fats, and fatty acids rank high among bile lipids. Lecithins (phosphatidylcholines, PCs) are the major representatives of bile phospholipids. They are synthesized in the liver from the same components as plasma PCs; however, they differ from the latter in the higher content of palmitic acid[7]. The human bile concentration of free fatty acids and α-monoglycerides is small. Human cystic bile shows small quantities of diglycerides and is virtually free of triglycerides.

The electrolyte content of bile is similar to that of plasma. The major cations are sodium, potassium, and calcium; the anions are chloride and bicarbonate. The bile content of sodium is about 10 times higher than that of potassium. Excretion of sodium, potassium, and calcium into the bile is closely related to the rate of metabolic processes in the liver and depends on its functional state and on the content of salts in the body. The bile concentration of anions is 5-15 times smaller than that of cations.

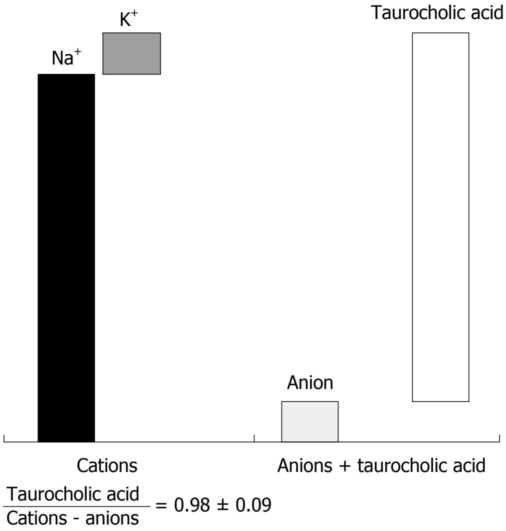

Figure 1 The bile content of anions, cations and taurocholic acid.

A deficit of anions is compensated for by taurocholate (Figure 1). Bile contains a considerable quantity of phosphorus, magnesium, iodine, iron, and copper. The relative proportions of the major bile components are distributed in the following order: bile acids (67%), phospholipids (22%), proteins (4.5%), cholesterol (4%), and bilirubin (0.3%). Among the bile acids, the primary bile acids, cholic and chenodeoxycholic acids in a ratio of 1:1, account for about 50%. These are followed by the secondary bile acids, deoxycholic and lithocholic acids, as well as ursodeoxycholic and sulfolithocholic acids in the decreasing order.

The use of color cathode-luminescence scanning electron microscopy (CCL SEM) has made it possible to study the qualitative composition of dehydrated human bile and its components in the native state. CCL SEM provides video information about the structural pattern of bile and its chemical composition. Loginov *et al*[8] were the first to show the earlier unknown phenomenon of cathode autoluminescence of non-conjugated bilirubin, unesterified cholesterol, high-molecular-weight protein, and other organic compounds. The pictures (Figure 2) show that the amorphous powder mass of bilirubin is red-emitting (Figure 2A), crystal cholesterol luminesces blue (Figure 2B), and the green coloration tingled with red is predominant in the sample of protein (Figure 2C). Graphically, all three samples have chromaticity histograms in the form close to the Gaussian curve.

Examination of the dehydrated samples of normal bile by CCL SEM has ascertained the tree-type structure of precipitates (Figure 2D). The qualitative assessment of the samples suggests their balanced content of three ma-

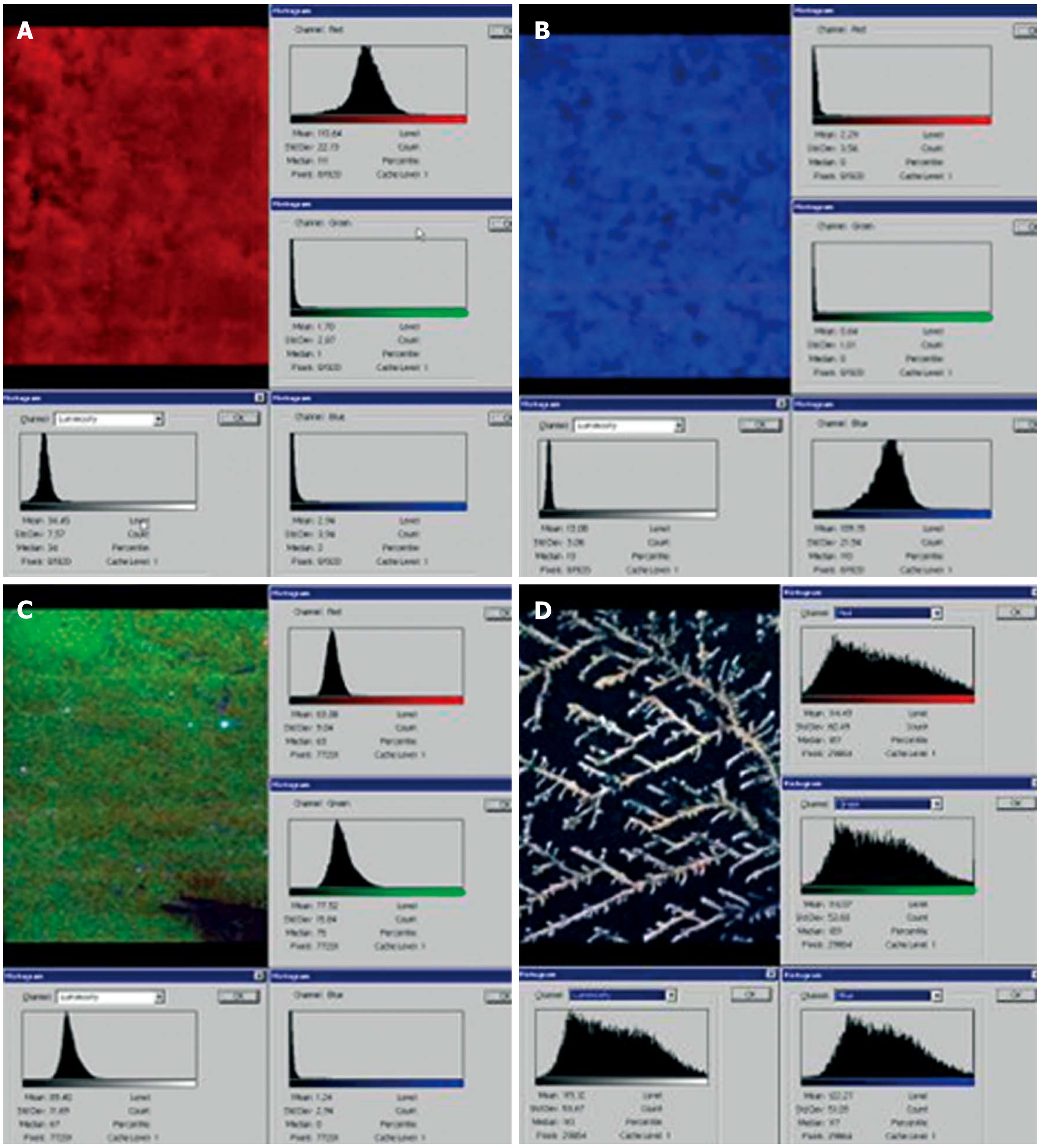

Figure 2 Color cathode-luminescence scanning electron microscopy images. Color cathode-luminescence scanning electron microscopy (CCL SEM) micro images of unconjugated bilirubin (A), unesterified cholesterol (B), high-molecular-weight protein (C), and dehydrated sample of normal human cystic bile (D).

jor bile components: bilirubin, cholesterol, and protein. Hepatocytes are the major epithelial cells in the liver, and are polarized. The polarized surfaces of hepatocytes consist of a basolateral domain facing the circulation and an apical domain that forms the bile canaliculus; the smallest branch of the biliary tree[9]. A major function of the liver is biliary secretion, which requires hepatocyte polarization. The mechanisms controlling hepatocyte polarization are only partially understood[10]. Structurally, they include cytoskeletal, tight junction and intracellular trafficking components. Recently, it was discovered that the major mammalian bile acid, taurocholate, accelerated polarity in primary rat hepatocytes. Taurocholate increases cellular cAMP and signals through an exchange protein activated by cAMP (EPAC)-Rap1-MEK-liver kinase B1 (LKB1)-AMP-activated protein kinase (AMPK) pathway for its polarity effect. Fu *et al*[9] have discussed possible mechanisms for how taurocholate affects different cell polarity factors, particularly AMPK, and thereby regulates events that generate polarity.

Bile production starts in the intercellular bile canaliculi. The bile canalicular lumen is formed by the external hemileaflet of the apical part of the plasma membrane of the adjacent hepatocytes and the tight junctions located at the point of contact of the hepatocytes. The hepatocyte tight junctions separate the bile canalicular lumen from the hepatic blood system[11]. Tight junctions contain proteins, including occludin, claudin and zona occludens (ZO)-1 protein. The integrity of tight junctions depends on the presence of protein ZO-1 on the inner surface of the plasma membrane. The impaired integrity of the tight junctions is accompanied by regurgitation of

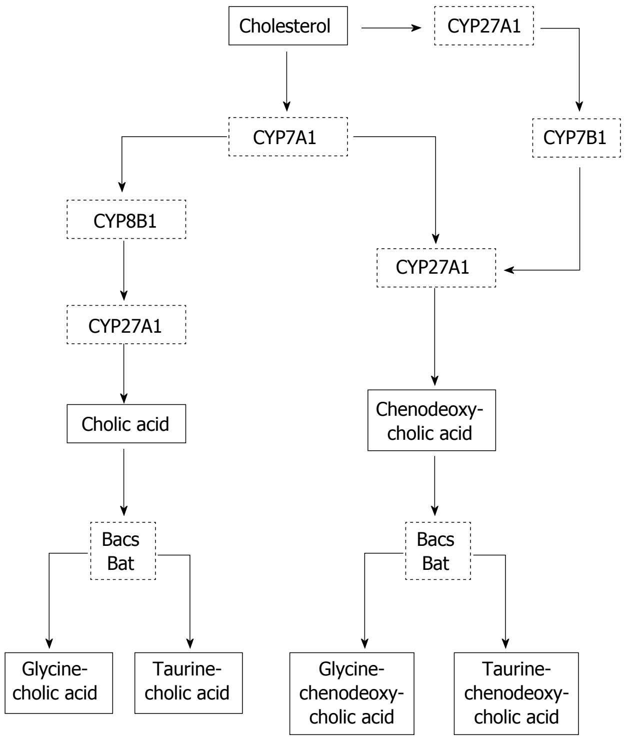

Figure 3 Bile acid synthesis. CYP7B1: 7α-hydroxylase; CYP7A1: Enzyme cholesterol-7α-hydroxylase; CYP8B1: Sterol 12 α-hydroxylase; CYP27A1: Mitochondrial sterol 27 hydroxylase.

canalicular bile into the sinusoids. Three individual domains, such as sinusoidal, lateral, and canalicular parts of the membrane, are arbitrarily identified in the hepatocyte plasma membrane. The differences in the lipid and protein composition of the three domains of the hepatocyte plasma membrane determine its functional polarity. The sinusoidal membrane is enriched with receptors, enzymes, and transport proteins. The lateral membrane of the hepatocyte is involved in intercellular interaction, but it contains no transport systems. Transport systems for bile acids, organic anions and cations, as well as the enzymes γ-glutamyltransferase, Mg^{2+}-ATPase, and alkaline phosphatase are located on the canalicular membrane. Cl^-/HCO_3^- exchangers and selective Cl^- channels are also identified[12]. Whether there are transport systems for glutathione and its conjugates in the apical membrane is still debated[13]. The release of glutathione S-conjugates from cells is an ATP-dependent process mediated by integral membrane glycoproteins[14].

The hepatocyte cytoplasm around the bile canaliculus contains cytoskeletal structures: microtubules, microfilaments, and intermediate filaments[15]. The microtubules take part in the mediated vesicular transport, secretion of lipids. The microfilaments determine canalicular contractility and motor activity. The intermediate filaments form a network between the plasma membranes, nucleus, intracellular organelles, and other cytoskeletal structures. The canaliculus is the least branch of a biliary tree and has a diameter of approximately 1 μm.

BIOSYNTHESIS OF BILE ACIDS

In 1848 Strecker discovered bile acids[16,17]. In chemical structure, they belong to a group of steroids and are cholanic acid derivatives. Bile acids are the end metabolic product of cholesterol and one of the most important routes of its elimination from the body[18-20]. Bile acid synthesis occurs through two pathways: the classic (neutral) pathway or the alternative (acidic) pathway[21,22]. The liver is the only organ that has all 14 enzymes required for *de novo* synthesis of two primary bile acids in humans, cholic acid (CA; 3α, 7α, 12α-trihydroxy-cholanic acid) and chenodeoxycholic acid (CDCA; 3α, 7α-dihydroxy-cholanic acid)[23].

The biosynthetic pathway includes a number of successive enzymatic conversions associated with the oxidation of cholesterol in the smooth endoplasmic reticulum and the shortening of its side chain in mitochondria. The production of bile acids involves restoration of a double bond in cholesterol, C-3 inversion to give rise to a 3α-OH-group, further α-hydroxylation of either only a 7-carbon atom or 7- and 12-carbon atoms, as well as β-oxidation of the side chain of cholesterol. Cytochrome P450 is involved in all oxidative reactions. The classic bile acid biosynthetic pathway is initiated by enzyme cholesterol-7α-hydroxylase (CYP7A1) (Figure 3)[20]. The enzyme CYP7A1 of smooth endoplasmic reticulum is a limiting link in the synthesis of bile acids[20]. The activity of this enzyme is regulated by the quantity of intestinally absorbed bile acids, other than cholesterol[20,24]. Numerous studies have demonstrated that bile acids, steroid hormones, inflammatory cytokines, insulin, and growth factors inhibit *CYP7A1* transcription through the 5'-upstream region of the promoter[25-30]. Sterol 12 α-hydroxylase (CYP8B1) is required for synthesis of CA. Mitochondrial sterol 27 hydroxylase (CYP27A1) catalyzes sterol side chain oxidation, after which cleavage of a three-carbon unit in the peroxisomes leads to formation of a C24 bile acid. Cholesterol is converted to two primary bile acids in human liver, CA and CDCA. Key regulated enzymes, CYP7A1, CYP8B1, CYP27A1, and 7α-hydroxylase (CYP7B1) expressed, in the pathways are indicated. CYP7A1 initiates the classic (neutral) bile acid biosynthetic pathway in the liver. CYP27A1 initiates the alternative (acidic) pathway in the liver and macrophages. CA and CDCA are conjugated to glycine (G) and taurine (T). Bile acid: CoA synthase (BACS) and bile acid: amino acid transferase (BAT) are two key enzymes involved in amino conjugation of bile acids[20].

An alternative (acidic) pathway is initiated by CYP27A1, which in addition to the liver is expressed in macrophages and most other tissues, and may contribute significantly to total bile acid synthesis (Figure 3). Other minor pathways initiated by 25-hydroxylase in the liver and 24-hydroxylase in the brain also may contribute to bile acid synthesis. A nonspecific CYP7B1 expressed in all tissues is involved in the generation of oxidized metabolites (oxysterols), which may be transported to the liver and converted to CDCA[20]. Farnesoid X receptor (FXR) plays a central role in the regulation of bile acid synthesis, excretion, and transport[31,32]. FXR inhibits *CYP7A1*[33], *CYP8B1*[34], and *CYP27A1*[35] transcription. It has been reported that peroxisome proliferator activated receptor (PPAR)α plays a role in the regulation of bile acid synthesis[36]. Bile acids induce *PPARα* transcription *via* induction of FXR[37].

Di- and tri-hydroxycholanic primary bile acids are produced from cholesterol during biosynthesis of bile acids in humans. Under normal conditions, 200-600 mg primary bile acids is formed daily[20,38]. Bile acid synthesis increases in the morning regardless of food intake[39]. In humans, bile acid synthesis exhibits a diurnal rhythm with two peaks around 15:00 and 21:00 h[20]. Bile acids lost in the feces (0.2-0.6 g/d) are replenished by *de novo* synthesis in the liver to maintain a constant bile acid pool. Hepatic conversion of cholesterol to bile acid balances fecal bile acid excretion and this process represents a major route for elimination of cholesterol from the body[40,41].

Bile acids are physiological detergents that generate bile flow and facilitate intestinal absorption and transport of lipids, nutrients and vitamins[20]. Bile acids, end products of the pathway for cholesterol elimination, are required for dietary lipid and fat-soluble vitamin absorption and maintain the balance between cholesterol synthesis in the liver and cholesterol excretion[21].

CONJUGATION OF BILE ACIDS

Under physiological conditions, free bile acids are not frequently found in bile. Newly synthesized bile acids conjugate with glycine or taurine, by giving rise to bile salts: (chenodeoxy-)cholylglycine or (chenodeoxy-)cholyltaurine (Figure 3). The physiological significance of bile acids conjugation is that their salts are more polar compounds than free bile acids. Conjugation of bile acids to glycine or taurine decreases bile acid toxicity and increases their solubility that benefits secretion into bile. Conjugated bile acids have a smaller critical concentration for micellar formation[42]. The taurine conjugates of bile acids are more polar compounds than their glycine conjugates[43]. Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in humans and plays an important role in several essential biological processes such as bile acid conjugation, maintenance of calcium homeostasis, osmoregulation and membrane stabilization[44]. Taurine is efficient at reducing plasma and liver cholesterol concentrations. The cholesterol-lowering effect of taurine is involved in the regulatory mechanism of cholesterol and bile acid homeostasis that is mediated by CYP7A1, which has become a biomarker for cholesterol metabolism and is itself also regulated by several factors and nuclear receptors[45].

The C-24 conjugation of free cholanic acids with glycine or taurine is accomplished by hepatocyte acyltransferases. The reaction proceeds in two steps with the participation of ATP and in the presence of Mg^{2+}. BACS and BAT are involved in amino acid conjugation of bile acids[20]. FXR stimulates bile acid conjugation by inducing expression of genes encoding BACS and BAT, which also are induced by hepatocyte nuclear factor (HNF) 4α[46]. Thus, FXR and HNF4α may coordinately regulate bile acid synthesis and conjugation.

The normal human ratio of the glycine to taurine conjugates of bile acids is 3:1. The ratio of the glycine/taurine conjugates may be altered under the influence of alimentary and hormonal factors, in some liver diseases. The presence of unconjugated bile acids in the bile most frequently is a result of hepatic disease.

In the intestine, glyco- and tauro-conjugated CA and CDCA are deconjugated, and 7α-dehydroxylase activity in bacterial flora removes a 7α-hydroxy group to form secondary bile acids deoxycholic acid (DCA; 3α,12α-dihydroxy) and lithocholic acid (LCA; 3α-monohydroxy), respectively[20].

Bile acids may be also exposed to glucuronidation or sulfation, which results in a reduction of their toxic properties and promotes their urinary and fecal excretion[43,47-49]. A small amount of bile acids circulated to the liver is sulfoconjugation at the 3-hydroxy position by sulfotransferase (SULT2A1) and rapidly secreted into bile. Sulfation is the major pathway for detoxification of extremely hydrophobic bile acids in humans[50]. Details of bile acid chemistry, biology, physiology, and synthesis have been reviewed recently[51,52].

BILE PHOSPHOLIPIDS

Phospholipids are a heterogeneous group of substances. They contain fatty acids with a varying length of carbon chain. Phospholipids are an important component of cell membranes, plasma, and bile. In all mammalian species, including humans, bile phospholipids are represented solely by a mixture of PC (lecithin) molecules. The fatty acids of bile PC molecules greatly differ from those of lecithin of plasma and liver tissue. Depending on fatty acid residues at C1 and C2 of the glycerine molecule that is part of PC, there are a variety of lecithins. Human bile predominantly contains 1-palmitoyl, 2-linoleyl, and 1-palmitoyl, 2-oleoyl PCs. This lecithin subpool represents nearly 5% of the total pool of hepatic PCs. The lecithins destined for bile secretion have been shown to be apparently completely synthesized *de novo*[53]. Selection of certain types of PC seems to occur during bile formation[7]. Bile salts and primarily PC are the main organic solutes in bile, and play a crucial role in cholesterol and dietary lipid solubilization. Bile salts and phospholipids in concert increase the biliary solubility of cholesterol by > 1 million-fold, thereby permitting entry of hepatocellular cholesterol into bile[54]. Hepatic PC biosynthesis is likely to be regulated by bile salts. Biliary lipid secretion is driven by bile salts, the primary metabolites of cholesterol. There are multiple data showing that the biliary secretion of PC (as cholesterol secretion) depends on that of bile acids[55,56]. It has also been shown that the secretion of bile phospholipids is not only associated with that of bile acids, but is regulated by the amount and type of bile

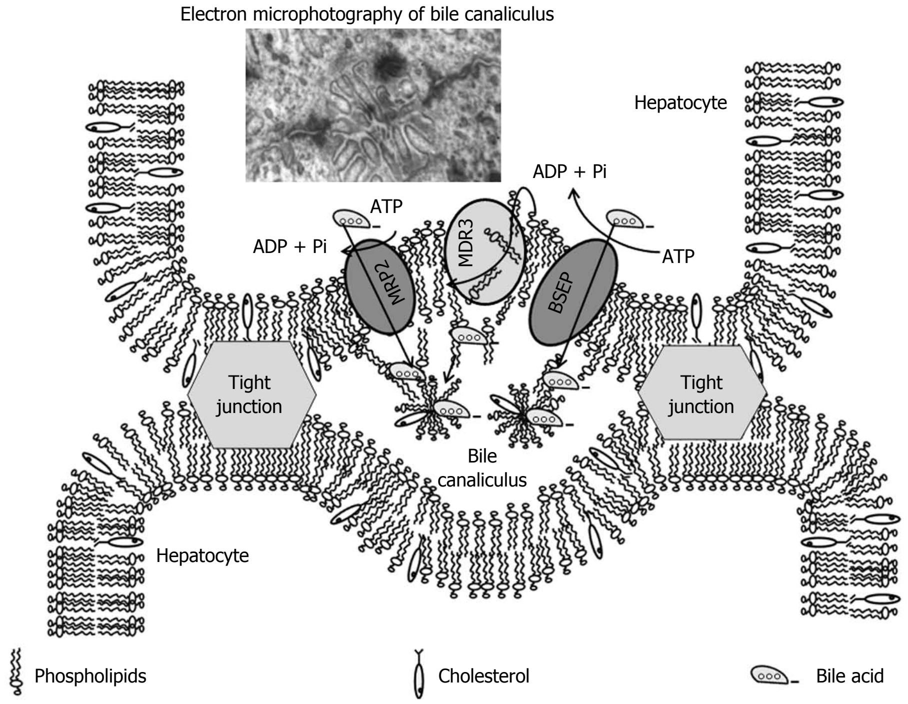

Figure 4 Proposed model for lipid secretion into bile[58]. BSEP: Bile salt export pump; MDR3: Multidrug-resistance protein 3; MRP2: Multidrug-resistance-associated protein 2; ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; Pi: Inorganic phosphate.

salts passing through the hepatocytes[7,57].

The use of electron microscopy has demonstrated that phospholipids are secreted as vesicles into bile, and that vesicle secretion is almost wholly dependent upon MDR3 P-glycoprotein function[58]. Biliary-specific PC molecules are recruited initially from intracellular sources, predominantly the endoplasmic reticulum[59]. A proposed mechanism for PC delivery to the canalicular plasma membrane involves monomeric transfer *via* binding to the cytosolic PC transfer protein (PC-TP)[60]; a process that is stimulated by low concentrations of bile salts typical of the hepatocytic cytosol. Alternatively, biliary lipid may arrive at the canalicular membrane *via* intracellular vesicle transport[1].

Biliary PC molecules are translocated from the endoplasmic to the exoplasmic hemileaflet by the action of a transmembrane translocator[61].

MDR3 P-glycoprotein is thought to translocate PCs from the internal to the external hemileaflet of the hepatocyte canalicular plasma membrane; possibly generating microdomains of the more fluid biliary-type PCs. Luminal bile salts, secreted as monomers through the action of bile salt transporters (cBATs), interact preferentially with these microdomains to promote vesiculation of the membrane external hemileaflet; possibly involving nonlamellar phase transitions. Cholesterol, which can freely flip between the internal and external hemileaflets, may be released into bile either by diffusing laterally into nascent vesicles, or by bile-salt-mediated transfer through the aqueous phase. The model is based on data from the study[59] and published work[1,62,63].

Crawford *et al*[58] proposed the concept that biliary PC molecules are flipped from the internal to the external hemileaflet of the hepatocyte canalicular membrane by the action of MDR3 P-glycoprotein, followed by release of PC vesicles from the external hemileaflet into bile (Figure 4). Formation and detachment of unilamellar vesicles from the canalicular membrane is presumably mediated by the detergent action of luminal bile salts.

Vesiculation of the external hemileaflet provides an explanation of how luminal bile salts can extract large quantities of phospholipid on the basis of their detergent action, without disrupting the integrity of the detergent-resistant canalicular plasma membrane. This remarkable mechanism for selective secretion of membrane phospholipids appears to be uniquely adapted to the detergent environment of the bile canaliculus.

BILE MICELLES

Bile lecithins, bile acid salts, and cholesterol are amphiphiles molecules (Figure 5). In this connection, in the water medium, such as bile, these compounds cannot exist in the monomolecular form and they generate micellar or lamellar structures.

According to the inclusions into the lipid complex, simple micelles (PC + cholesterol), up to 3 nm in size, mixed (PC + cholesterol + bile acids), 3-6 nm in diameter, and vesicles (PC + cholesterol + bile acids), 25-130 nm in diameter, are identified (Figure 6).

They can all contain amphiphilic proteins on their surface. Lipid molecular inclusions in the micelles of bile acids and the formation of mixed micelles are the main form of interaction of bile acids and lipids in the bile.

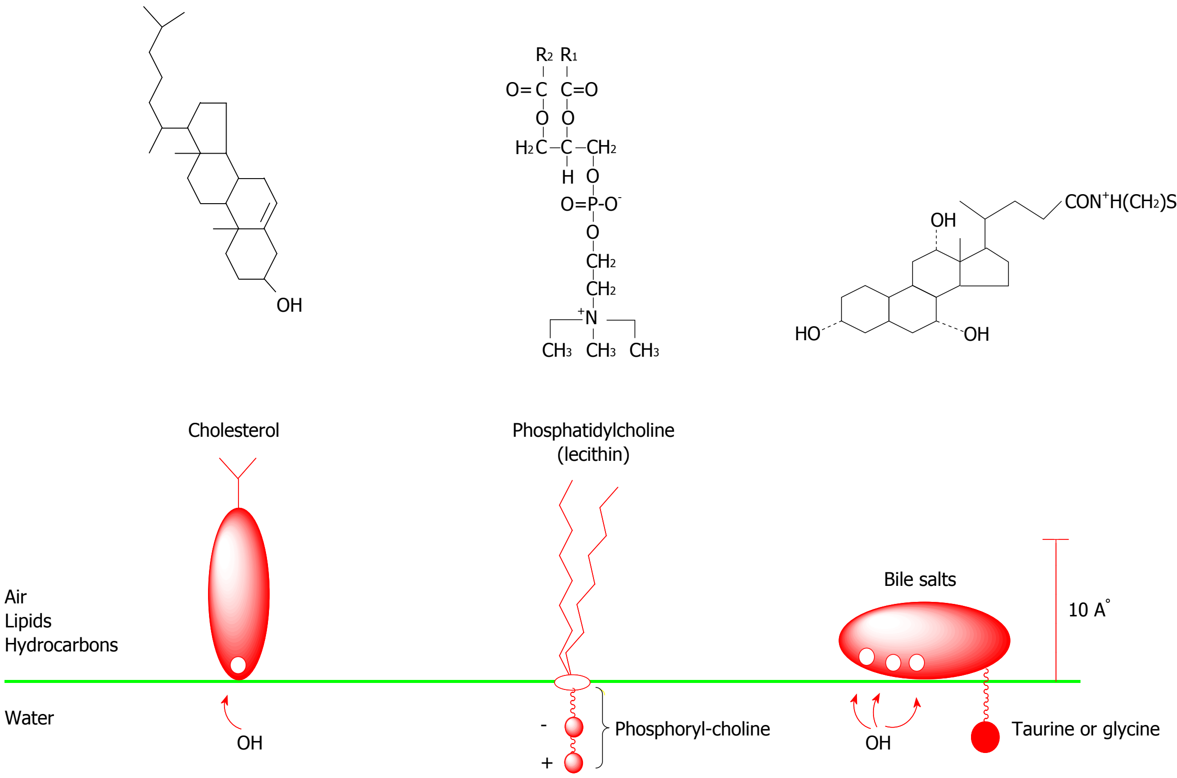

Figure 5 Chemical formulas and simple images of cholesterol, lecithins, and bile salts on a boundary of two phases-water: lipids (air, hydrocarbons).

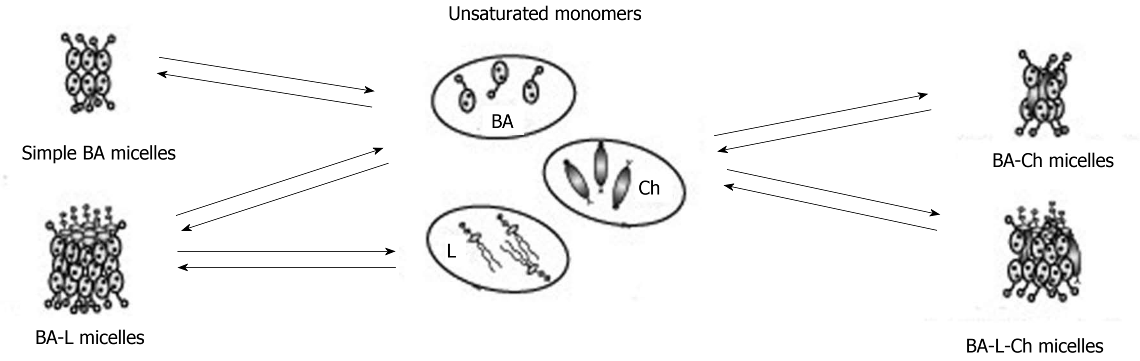

Figure 6 Formation of micelles structures in the system containing bile acids, lecithin, and cholesterol. BA: Bile acids; Ch: Cholesterol; L: Lecithin.

By forming mixed micelles, bile acids along with lecithin provide cholesterol solubilization. Bile salts as detergents form micelles when the critical concentration of micellar formation (CCM) is achieved. CCM depends on the type of bile salts and their hydrophobicity. Based on their hydrophobic properties, bile acids may be arranged in the following order: deoxycholate > chenodeoxycholate > cholate > ursodeoxycholate[64]. Hydrophobic, but not hydrophilic, bile acids are efficacious endogenous ligands of the nuclear receptors FXR (NR1H4), pregnane X receptor (PXR; NR1I2), and vitamin D receptor (VDR; NR1I1) that play critical roles in the regulation of bile acid synthesis and metabolism[20].

A mixture of bile acids, lecithin, and cholesterol at certain molecular ratios is able to form lamellar liquid-crystalline structures[64]. Vesicles are monolamellar globules that consist of phospholipids and bile acids. At a certain concentration (about 0.1 mmol/L), phospholipids as amphiphiles have been found to produce enclosed globules, in which the polar heads are directed outward, and the nonpolar "tails" are inside. This particle is capable of forming many substances, including protein, and cholesterol, by providing solubilization of the latter by bile acids. Vesiculation is largely determined by the hydrophilic-hydrophobic balance of bile acids and the content of cholesterol[65]. The proportion of bile-mixed micelles and vesicles depends on the concentration and composition of bile acids. The most important feature of phospholipid masses is their ability to change their consistency from crystalline gel to liquid-crystalline state depending on environmental conditions. The liquid-crystalline states are typified by the structural order peculiar to true crystals with the preserved mobility of the molecules observed in solutions. The fusion of monolamellar vesicles may give rise to multilamellar vesicles. The vesicles are a rather stable formation and together with micelles play a pivotal role in the solubilization and transport of cholesterol into the bile[24].

Lipid vesicles 60-80 nm in diameter have been documented repeatedly by electron microscopy and quasielastic light scattering of freshly secreted hepatic bile[1,58]. Microscope laser light scattering of bile canaliculi in isolated rat hepatocyte couplets also detects intraluminal vesicles of similar size; the numbers of which increase upon cellular incubation with bile salts[66].

Acid-dependent and acid-independent bile fractions are identified[67]. The former is associated with the synthesis and active transport of bile acids in the hepatocytes. The volume of the resultant bile is linear with the concentration of bile acids, caused by the osmotic effect of bile acid anions, and Sperbet[68] first argued it in 1959. The bile composition greatly varies in the bile canaliculi due to the increased constant volume of water and bicarbonates irrespective of the secretion of bile acids. This acid-independent bile fraction is regulated by an active Na^+ transport. Its physiological role is to dilute and excrete an acid-dependent fraction[69]. Further bile formation occurs in the bile ducts because a fluid enriched with bicarbonates and chlorides is produced in the latter. This process is under the control of secretin (80%) and other gastrointestinal hormones. The volume of the water secreted in the ducts depends to greater degree on the active transport of bicarbonates[70].

ENTEROHEPATIC CIRCULATION OF BILE ACIDS

The bile acids synthesized in the hepatocyte participate in the human body, in the so-called enterohepatic circulation. Conjugated bile acids are secreted from the hepatocytes into the bile canaliculus by canalicular bile salt export pump (BSEP, ABCB11). Some bile acids secreted in the bile duct are reabsorbed in the cholangiocytes and recycled back to hepatocytes (the cholangiohepatic shunt)[20]. The bulk of bile acids secreted are stored in the gallbladder. After each meal, gallbladder contraction empties bile acids into the intestinal tract. Bile acids are mixed up with food masses (together with other digestive secretions), and take an active part in the processes of fat and fat-soluble vitamins metabolism and absorption.

Fatty acids and monoglycerides formed from neutral fats with participation of bile acids and of lipases in the upper parts of the small intestine are absorbed by enterocytes as lipoid-biliary complexes. These complexes disintegrate in the enterocytes. Released monoglycerides and fatty acids are used by the cells as energy and building materials or are transported to the basolateral membrane of the enterocytes and enter the portal venous system. Bile acids may return to the intestine and continue to participate in metabolism and absorption of fat and fat-soluble vitamins. As bile acids are moving through the intestine they may participate 4-6 times in the transportation of monoglycerides and fatty acids in enterocytes.

In the small intestine, bile acids are absorbed by both passive and active mechanisms[71]. Although passive absorption occurs down the length of the intestine, active absorption of bile acids is restricted to the ileum[72,73]. Passing through the intestinal tract, some bile acids are reabsorbed in the upper intestine by passive diffusion, but most bile acids (95%) are reabsorbed in the distal ileum by apical sodium-dependent bile acid transporter (ASBT) located in the brush border membrane[71]. In humans and all other vertebrates examined to date, the ileal epithelium has developed an efficient transport system for the active reclamation of bile acids[51]. Approximately 95% of the bile acids secreted into bile are derived from the recirculating pool[71]. Bile acids are transdiffused across the enterocytes to the basolateral membrane where the organic solute transporter and heterodimer (OST/OST) effluxes bile acids into the portal blood circulation[74,75], transports them to the sinusoids, where they are taken up by Na^+-dependent taurocholate cotransport peptide (NTCP) into hepatocytes[20]. The bulk of bile acids are absorbed by hepatocytes into the liver, and again excreted into the bile. Later, this enterohepatic cycle recurs. Hepatocytes are known to secrete as much as 90% of the bile acids that have returned into the cells during enterohepatic circulation, and about 10% of the newly synthesized bile acids[20]. A bile acid pool of about 3 g is recycled 4-12 times a day[71]. At a fundamental level, the bile acid enterohepatic circulation can be viewed as a series of storage chambers (gallbladder and small intestine), valves (sphincter of Oddi and ileocecal valve), mechanical pumps (canaliculi, biliary tract, and small intestine), and chemical pumps (hepatocyte and enterocyte transporters)[71].

The enterohepatic circulation of bile acids serves as an important physiological route for recycling bile acids and absorption of nutrients, as well as regulation of whole-body lipid metabolism[20]. Enterohepatic circulation ensures a continuous supply of bile acids to be used repeatedly for lipid absorption during the digestion of a single meal or multiple meals throughout the day[76]. Efficient intestinal reabsorption and hepatic extraction of bile acids also enables effective recycling and conservation that largely restricts these potentially cytotoxic detergents to the intestinal and hepatobiliary compartments.

FXR plays a key role in the control of enterohepatic circulation of bile acids. FXR induces the expression of BSEP in the canalicular membrane, which is the driving force for bile formation. FXR inhibits *Ntcp* transcription by SHP-dependent inhibition of retinoid X receptor/retinoic acid receptor induction of *Ntcp*[77]. Thus, FXR plays a critical role in the coordination of bile acid synthesis, biliary bile acid secretion, intestinal bile acid reabsorption and secretion, and bile acid uptake into hepatocytes.

Hepatocytes hold a central position in the enterohepatic circulation of bile acids, with which their highest (as compared to other cells) content of cholanic acids is associated.

Molecular mechanisms of hepatocyte bile secretion

Hepatocyte secretion of bile is the passive transhepatocellular filtration of water and electrolytes (Na^+, K^+, and Cl^-) from blood into the bile canalicular cavity, which is caused by the osmotic gradient between the bile and blood, which results from active transport of organic and inorganic anions from blood and hepatocytes to the bile canaliculi. The ionic transport systems that govern ionic and electrical gradients onto the membrane surface (Na^+/K^+, Na^+/H^+, Na^+/HCO_3^-, Cl^-/HCO_3^-, and Na^+/Ca^{2+}) are the basis for the mechanism of targeted bile secretion[78]. The transport of bile salts, organic anions and cations, bilirubin and other substances from the portal blood into the biliary system is accomplished through the action of an array of transporter proteins in the hepatocytes[79].

The hepatocytes are polar cells in which the transport of osmotically active bile components has a strict direction from the sinusoidal (basal) surface to its canalicular (apical) part. There is evidence on the importance of taurocholic acid in the directed transport of substances in the liver cells[44]. This vectorial trans-hepatocellular movement of bile acids is a remarkably concentrative transport process that is driven by a distinct set of primary (ATP-dependent), secondary (Na^+ gradient-dependent), and tertiary (OH^- or HCO_3^--dependent anion exchange) transport systems at the sinusoidal and canalicular plasma membranes[80,81]. Bile secretion is a highly regulated process. To maintain this process, liver cells must transport bile acids efficiently from the portal blood into bile. ATP binding cassette (ABC) transporters[57] mediate transport of bile salts. Expression of these transporters is regulated in a coordinate fashion by a set of nuclear hormone receptors explaining the old observation of coupling between bile salt secretion and biliary lipid secretion. Over the past two decades, there has been significant progress toward identifying the individual membrane transporters and unraveling their complex regulation[74]. The hepatocytes are polarized cells that express differential transport systems in their plasma membrane domains. Molecular cloning has identified most of these transport proteins, and their transport properties characterized by functional studies. The NTCP, the bile salt export pump (BSEP), the ASBT, and the organic solute transporter OSTα-OSTβ, the major bile acid transporters that control the fate of bile acids, through either absorption and enterohepatic cycling or excretion and elimination from the body.

These transporters play a key role in the vectorial transfer of solutes and water from sinusoidal blood into the bile, thus contributing to bile formation and the biliary excretion of various xenobiotics. In the liver and intestine, transporters play a critical role in maintaining the enterohepatic circulation and bile acid homeostasis[71,82].

Bile acids determine the secretion of an acid-dependent bile fraction. The transport of bile acids from the sinusoidal space to the bile canaliculus comprises their penetration across the sinusoidal and canalicular membranes and movement inside the hepatocytes. The molecular and metabolic processes occurring on the hepatocyte sinusoidal membrane involve: (1) hepatocyte absorption of bile acids and proteins from blood; and (2) transmembrane transfer of inorganic ions. The principal intracellular molecular and metabolic processes determining the mechanisms of hepatocyte bile secretion are: (1) biosynthesis and conjugation of bile acids; (2) hydroxylation and conjugation of bile acids entering the hepatocytes from the blood; and (3) transfer of bile acids and proteins from the perisinusoidal to pericanalicular area of the hepatocytes.

The molecular mechanisms of hepatocyte canalicular membrane function ensure the transmembrane transfer of bile acids, proteins, inorganic and organic cations and anions into the bile canalicular cavity.

Molecular physiology of the transporting systems of the hepatocytic sinusoidal membrane: The specific feature of transport of bile acids from blood to the hepatocyte across the sinusoidal membrane is to overcome the high gradient of their concentration and electrical potential. Hepatocytes absorb bile acids from the blood by the bile acid carriers, which are integral hepatocyte sinusoidal membrane proteins[78] (Figure 7).

Liver sinusoids possess a specific architecture that allows passage of organic compounds bound to albumin through endothelial fenestrae into the space of Disse, from where they can be taken up by the sinusoidal transport systems of the hepatocytes[83,84].

The bulk (about 85%) of the bile acids are present in plasma as a complex of plasma proteins (mainly, albumin)-bile acid, therefore, it has been shown that albumin performs its inherent transport function and contributes to the interaction of bile acids with specific sinusoidal membrane receptors in the mechanisms of hepatocyte absorption of bile acids. The bile acids in sinusoidal blood are efficiently taken up by hepatocytes from Disse's space despite being highly albumin bound, due to the existence in the basolateral membrane of transporters[85,86]. The powerful techniques of molecular biology have enabled gene cloning of the transporters involved in biliary secretion and the enterohepatic circulation of bile acids. This has permitted elucidation of their function as well as their regulation by nuclear receptors[87]. The enterohepatic circulation results from efficient ileal absorption, and is highly regulated at two sites. In the hepatocytes, biosynthesis of bile acids is regulated by negative feedback by the nuclear receptor FXR as well as by cytokines and by a peptide (FGF-19) liberated by bile acids from the ileal enterocytes. In the ileal enterocytes, bile acid reclamation is regulated by negative feedback by FXR and other nuclear receptors. BSEP mediates uphill canalicular bile acid secretion[87]. The plasma membranes of hepatocytes have been found to contain the proteins that selectively bind bile acids[87]. This uptake is carried out against an electrochemical gradient, is saturable[88], and depends on the structure of the bile acids. It is more

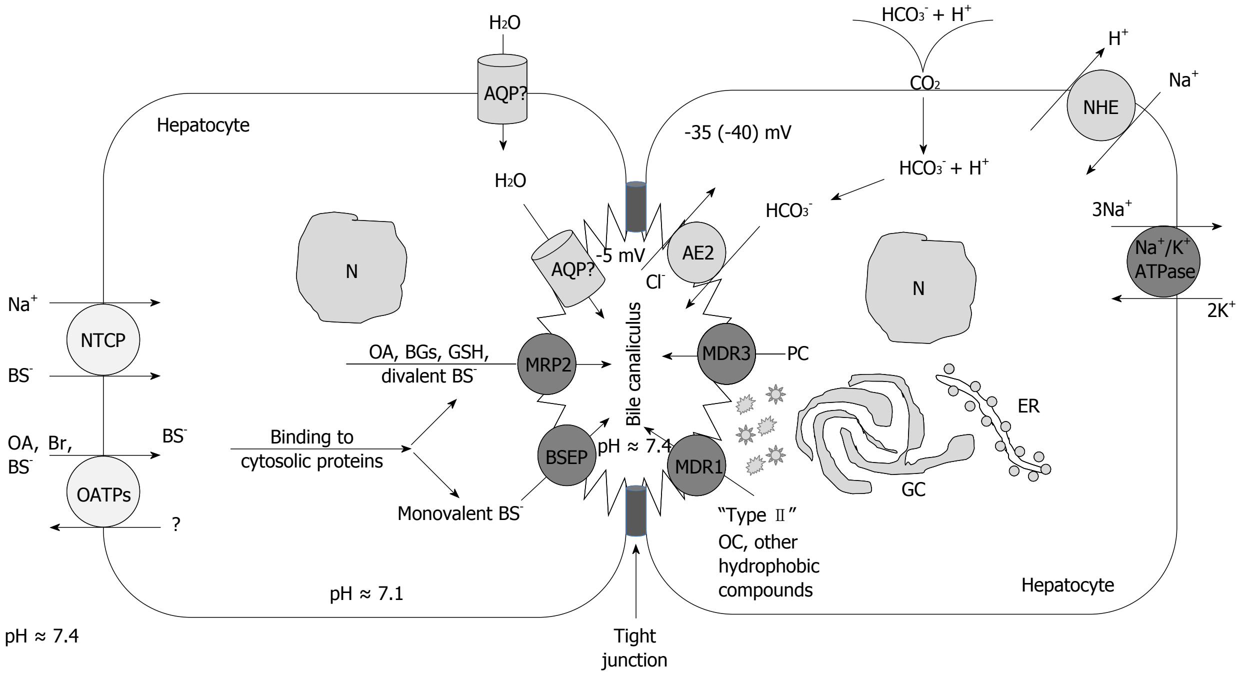

Figure 7 Mechanisms of the transport of bile acids, water, and electrolytes through the hepatocyte (transcellular pathway) and intercellular space (paracellular pathway). Localization and function of sinusoidal and canalicular hepatocellular transporters. The Na^+-dependent sinusoidal uptake of bile salts (BS) is mediated by Na^+-dependent taurocholate cotransport peptide. The Na^+-independent hepatic uptake of organic anions (OA^-), BSs and type Ⅱ organic cations (OC^+) is mediated by members of the OATP family. Sinusoidal uptake of type I OC^+ is mediated by OCT1. Transport across the canalicular membrane is driven mainly by ATP-dependent export pumps. MDR1 mediates canalicular excretion of amphiphilic type Ⅱ OC^+ and other hydrophobic compounds. MDR3 functions as a PC flippase. BSEP mediates apical excretion of BSs. MRP2 transports non-bile-salt organic anions, such as bilirubin glucuronides (BGs), GSH, and sulfated/glucuronidated bile salts. Canalicular transport of HCO_3^- is mediated by the Cl^-/HCO_3^- exchanger AE2. AQP9 and AQP8 are involved in the transport of water across the sinusoidal and the canalicular membrane, respectively. The nature of the water channels in human liver has been characterized[84]. GC: Golgi apparatus (complex); ER: Endoplasmic reticulum; N: Nucleus.

efficient for trihydroxy- than for dihydroxy-bile acids and for conjugated more than for unconjugated bile acids[89]. Transporters on the basolateral membrane, which faces the space of Disse, are responsible for the uptake of bile salts and organic anions[79]. The liver cells have an active transport system to transfer bile acids from the blood to the hepatocytes. Basolateral uptake transporters can be divided into Na^+-dependent and Na^+-independent systems. The NTCP, the main Na^+-dependent bile acids transporter[90-92] is only expressed in the basolateral membrane[93]. NTCP, (gene name SLC10A1) is the founding member of the SLC10 family of solute carrier proteins, which includes two bile acid carriers (SLC10A1/NTCP and SLC10A2/ASBT), one steroid sulfate transporter (SLC10A6/SOAT), and four orphan carriers (SLC10A3, SLC10A4, SLC10A5, SLC10A7)[94-96]. Na^+-dependent uptake involves co-transport of solutes with Na^+. Moreover, the driving force is the energy of ATP generated by Na^+,K^+-ATPase. This enzyme is located on the sinusoidal and lateral membranes of hepatocytes[97,98] and undetectable on the canalicular (apical) part[99,100]. The active transport of bile acids ensures their continuous hepatocyte pumping from blood to bile, thus causing their low level in the blood flowing from the liver and in plasma as a whole. The properties of NTCP satisfy all the functional criteria for hepatocyte Na^+-coupled bile acid uptake[71,94]. The major physiological substrates of NTCP include all the major glycine- and taurine-conjugated bile acids[93,101-104]. Depending on the structure of the bile acid and NTCP, unconjugated bile acids are moderate or weak substrates[102,105,106] and sulfated bile acids appear to be only weakly transported[107]. The high level of NTCP expression at the sinusoidal membrane of hepatocytes and high affinity of NTCP for conjugated bile acids promote efficient extraction of bile acids from portal blood. Thus, NTCP functions to maintain the enterohepatic circulation of bile acids and maintain plasma concentrations at a minimum.

The uptake of bile acids by Na^+-independent mechanisms seems to be mediated by less-specific transporters, known as organic anion-transporting polypeptides (OATPs), which exchange these molecules for other anions, such as HCO_3^-, glutathione (GSH) or even other bile acids[89,90,108].

Four OATPs have been cloned and characterized from human liver: OATP1A2 (SLCO1A2/SLC21A3; formerly, OATP-A), OATP1B1 (SLC21A6; formerly, OATP-C or LST-1), OATP1B3 (SLC21A8; formerly, OATP-8) and OATP2B1 (SLC21A9; formerly, OATP-B). These transporters may take up bile acids (mainly unconjugated forms), endogenous OA- (thyroid hormones, monoconjugated bilirubin) and xenobiotic compounds (*e.g.*, toxins, drugs and food components)[109]. In contrast to NTCP, members of the OATP family expressed on the hepatocyte sinusoidal membrane, such as Oatp1a1 (Slco1a1), efficiently transport unconjugated or sulfated bile acids, suggesting that these carriers participate in the hepatic uptake of those bile acid species *in vivo*[89,102,110]. The heterodimeric protein OSTα/OSTβ is expressed at the basal membrane of hepatocytes and cholangiocytes[111]. This is a sodium-independent bile acid transporter that may play a role in bile acid efflux from hepatocytes toward blood when these compounds are accumulated under cholestatic conditions. Moreover, in cholangiocytes, in addition to playing a similar role, this transporter may also be involved in the cholehepatic shunting of bile acids.

Hepatocellular uptake of organic cations is mediated by two separate transport systems, which depends on the substrate molecular size[112]. Thus, small (type Ⅰ) organic cations are taken up by the organic cation transporter, OCT1/Oct1 (SLC22A1/Slc22a1), which is electrogenic in nature. In contrast, human OATP-A mediates the uptake of bulky (type Ⅱ) organic cations. In addition to conjugated and unconjugated bile salts, Oatps/OATPs accept other cholephilic compounds, including glucuronidated (and maybe unconjugated) bilirubin, exogenous organic anions (*e.g.*, sulfobromophthalein), leukotrienes, estrogen-conjugates (*e.g.*, estrone-3-sulfate or estradiol-17-β-d-glucuronide), thyroid hormones, mycotoxins, and numerous xenobiotics[92,113-115].

Molecular physiology of the intracellular processes underlying the hepatocyte secretion of bile: In order to explain the transit of bile acids from the sinusoidal membrane to the pericanalicular region, two different, not mutually exclusive, mechanisms have been proposed: (1) simple diffusion of bile acids bound to intracellular proteins[116]; and (2) vesicular transport of bile acids driven by cytoskeleton contractile activity[117,118]. Two arguments have been raised against the role of the second mechanism. One is that hepatic transit of labeled bile acids is too fast[118]. The second is that the baseline secretion of bile acids is not modified by microtubular disruption[117]. However, the overload of bile acids intensifies the vesicular trafficking from the Golgi complex to the pericanalicular zone[119], and under these circumstances the alteration in the functional integrity of the cytoskeleton results in impaired bile acid secretion[120] and subsequent cholestasis[121].

The quantity of ABC transporters in the apical membrane is regulated by the amount of biliary components available for secretion[122,123]. The regulated intracellular vesicular traffic of canalicular ABC transporters[123,124] is crucial for normal bile secretion. BSEP (formerly SPGE, a sister of P-glycoprotein) is the main, if not the only, canalicular bile acid transporter[125], and it is also located in subcanalicular vesicles that may act as an intracellular pool. It is therefore probable that the impaired secretion of bile acids observed in overloaded conditions is an indirect result of the distortion of the increased vesicular traffic of transporters to the canalicular membrane[120]. These and other studies[126,127] have established the actual role of vesicular trafficking in hepatocytes, and have demonstrated that specific vesicle trafficking machinery is required for membrane polarity. The overall functions based on hepatocyte polarity are not attributable to the mere presence of transporters in both poles of these cells[128] but also to their intracellular trafficking and temporary anchorage to the different hepatocyte membranes (Figure 7).

Molecular physiology of the transporting systems of the hepatocyte canalicular membrane: After traversing the cell by Fick's diffusion, cholephilic compounds mostly bound to high-affinity cytosolic proteins are excreted into the bile mainly by ATP-dependent pumps of the superfamily of ABC transporters; in particular those belonging to the family of multidrug-resistance proteins, MDR/Mdr, or to the family of multidrug-resistance-associated proteins, MRP/Mrp[57,71].

MDRs/Mdrs are members of the ABC superfamily that were originally described in cancer cell lines, where they confer resistance to therapeutic agents. Three gene products were identified in rodents, Mdr1a (Abcb1a), Mdr1b (Abcb1b) and Mdr2 (Abcb4), and two in humans, MDR1 (ABCB1) and MDR 3 (ABCB4). MDR1/Mdr1 functions as an efflux pump for a wide range of amphiphilic, bulky type Ⅱ cationic drugs, together with other hydrophobic compounds, such as endogenous and exogenous metabolites or toxins, steroid hormones, hydrophobic peptides and even glycolipids[113]. Two closely related but functionally distinct Mdr1 isoforms, mdr1a and mdr1b, are present in the murine but not human phenotype[129]. MDR3/Mdr2 functions as a flippase, which translocates PC from the inner to the outer hemileaflet of the canalicular membrane, followed by release of PC-containing vesicles from the outer hemileaflet into bile; a process facilitated by the detergent properties of luminal bile salts[58].

Monoanionic bile salts are excreted in the canalicular pole by the bile salt export pump (BSEP/Bsep; ABCB11/abcb11); another member of the MDR family[130]. In contrast, canalicular efflux of divalent, bipolar sulfated or glucuronidated bile salts is mediated by the multidrug-resistance-associated protein 2 (MRP2/Mrp2; ABCC2/Abcc2)[131,132]. This carrier is also engaged in the biliary excretion of many other organic anions, including glutathione-S-conjugates (*e.g.*, leukotriene C4 or sulfobromophthalein, among others), glucuronides (*e.g.*, bilirubin and estrogens), and reduced (GSH) and oxidized glutathione (GSSG) - the former with low affinity[133,134]. Both GSSG and GSH are major determinants of the so-called "canalicular bile-salt-independent bile flow"[135].

The canalicular membrane domain also contains the electroneutral anion exchanger 2 (AE2/Ae2; SLC4A2/slc4a2), which extrudes HCO_3^- by exchanging the anion for biliary Cl^-[136]. It functions to regulate intracellular pH when hepatocytes are exposed to an alkaline load[136]. In addition, AE2/Ae2 plays a role in bile flow generation, because HCO_3^- excretion is thought to be an additional primary driving force of the canalicular bile-salt-inde-

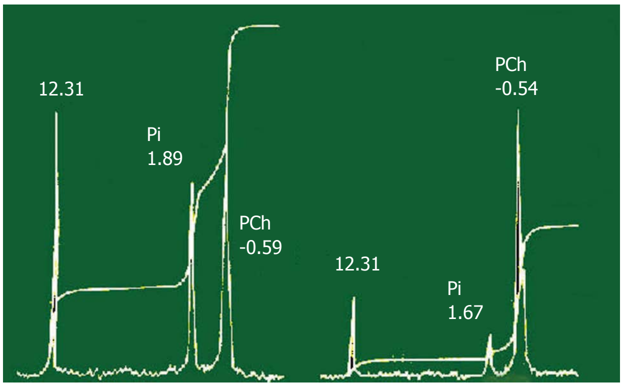

Figure 8 ^{31}P-NMR spectroscopy hepatic (left part of figure) and gallbladder (right part of figure) bile. Signals: 12,31 (standard); inorganic phosphate (P_i); phosphatidilcholine (PCh). PCh signal of gallbladder bile is increased with respect to such signals from hepatic bile. Signal of standard and inorganic phosphate of gallbladder bile are decreased with respect to such signals of hepatic bile. The concentration of lipids is increased by water absorption by gallbladder mucosa.

pendent bile flow[136,137]. Both in humans and rats, three transcript variants of AE2/Ae2 have been described: the full-length transcript AE2a/Ae2a, expressed from the upstream promoter in most tissues, and the alternative transcripts $AE2b_1$/$Ae2b_1$ and $AE2b_2$/$Ae2b_2$, expressed in a more tissue-restricted fashion (mainly in liver and kidney). $AE2b_{1/2}$/$Ae2b_{1/2}$ transcription is driven from overlapping promoter sequences within intron 2, which results in AE2/Ae2 protein isoforms with short N-terminal differences[138,139].

In water transporters, for a solute to drive blood-to-bile vectorial water transport primarily, resultant osmotic forces need to be associated with aquaporin (AQP)-mediated transcellular movement of water molecules from plasma to the bile canaliculus[140]. Both immunochemical and functional studies have demonstrated the constitutive expression of the water channel AQP9 at the basolateral membrane of rat hepatocytes, and the regulated expression of the water channel AQP8 at the hepatocellular canalicular membrane domain[140-142]. AQP8 is suggested to play a role in bile formation, facilitating the osmotic movement of water under choleretic stimulus[140,142]. AQP isoforms that mediate polarized water transport in human hepatocytes, if any, remain to be identified.

Gallbladder

The bile formed outside the digestive periods enters the gallbladder that performs two important functions: concentration of bile and its storage up to the evacuation into the duodenum. Minor quantities of bile acids (about 1.3%) are absorbed in the gallbladder walls. Micelles and vesicles enter the gallbladder where the concentration of lipids is increased by water absorption, which causes some physicochemical changes in the bile (Figure 8).

If the gallbladder functions well due to its contraction, all agglomerated vesicles and micelles with bile flow reach the duodenum. The gallbladder mucosa actively absorbs amino acids (this has been established by ^{35}S-labelled methionine) and an albumin bile protein fraction[143].

Thus, the gallbladder takes an active part in changing the composition of bile *via* reabsorption of its components into the blood and making the bile components circulate along the small circuit: liver-gallbladder-blood-liver.

The flow of bile is lowest during fasting, and a majority of that is diverted into the gallbladder for concentration. When chyme from an ingested meal enters the small intestine, acid and partially digested fats and proteins stimulate secretion of cholecystokinin and secretin. These enteric hormones have important effects on pancreatic exocrine secretion. They both are also important for secretion and flow of bile.

Cholecystokinin: The name of this hormone describes its effect on the biliary system-cholecysto = gallbladder and kinin = movement. The most potent stimulus for release of cholecystokinin is the presence of fat in the duodenum. Once released, it stimulates contractions of the gallbladder and common bile duct, resulting in delivery of bile into the gut.

Secretin: This hormone is secreted in response to acid in the duodenum. Its effect on the biliary system is similar to that seen in the pancreas: it simulates biliary duct cells to secrete HCO_3^- and water, which expands the volume of bile and increases its flow into the intestine.

Sphincter of Oddi

The sphincter of Oddi is located at the interface of the common bile duct and the main pancreatic duct at their confluence into the duodenum. Anatomical and immunohistochemical studies have indicated that the sphincter of Oddi is richly innervated with cholinergic, adrenergic, and peptidergic neurons.

The location of the sphincter of Oddi determines its function: to regulate the entry of bile and pancreatic juice into the duodenum and to prevent reflux of duodenal contents into the common bile duct and the main pancreatic duct. The sphincter coordinates the time and rate of secretion of about 3 L/d of bile and pancreatic juice into the duodenum[144]. Normally, the sphincter is characterized by considerable phase contractions throughout the interdigestive period.

Transport of bile acids along the portal venous system

Bile acid salts come from the intestine into the portal venous system. Bile acids with venous blood enter the liver where they are apparently completely (99%) absorbed by hepatocytes. And only a small quantity (about 1%) of bile acids enters the peripheral blood. In this connection, in healthy individuals the blood concentration of circulating bile acid salts is small.

In the hepatocytes, the secondary bile acids, deoxycholic and lithocholic, are subject to hydroxylation and they conjugate with glycine or taurine. Bile acids come as

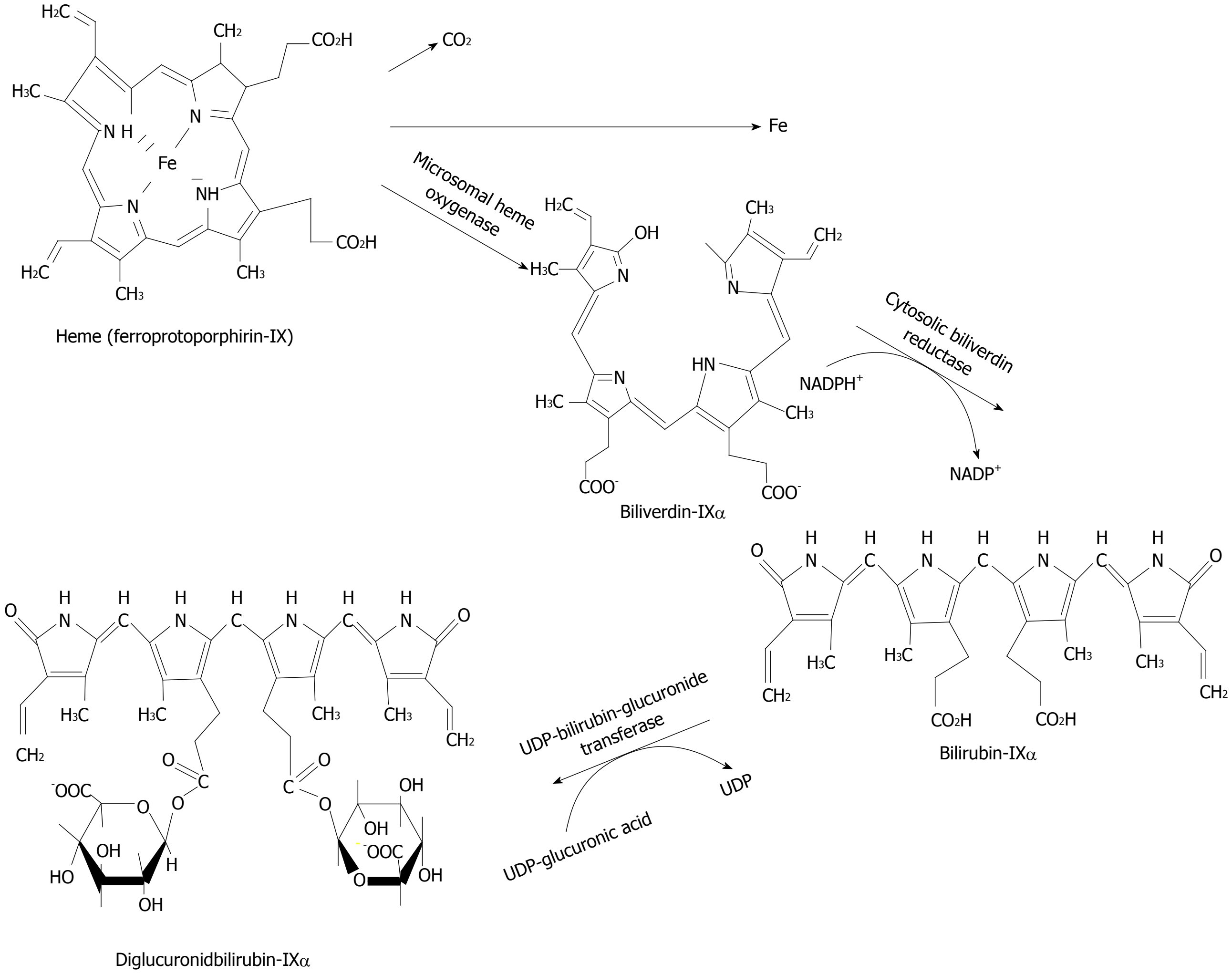
Heme (ferroprotoporphirin-IX)
CO2
Fe
Microsomal heme oxygenase
Biliverdin-IXα
Cytosolic biliverdin reductase
NADPH+
NADP+
Bilirubin-IXα
UDP-bilirubin-glucuronide transferase
UDP
UDP-glucuronic acid
Diglucuronidbilirubin-IXα

Figure 9 Formation of IXα bilirubin from heme.

conjugates from the liver into the bile again. The normal enterohepatic circulation of bile acids occurs 2-6 times daily depending upon the dietary regimen.

It has been shown that the normal kidneys are not involved in the excretion of bile acids from the body. Portal venous blood bile acids are bound by plasma proteins, mainly albumins[145] and, to a lesser extent, by α-and β-globulins. The data available suggest binding of blood bile salts to lipoproteins[146,147]. The bile acids are loosely bound to lipoproteins. In healthy individuals, bile acids are detectable in all classes of lipoproteins[147].

METABOLISM OF BILE PIGMENTS

Bilirubin is a principal bile pigment. Bilirubin is formed in the cells of the reticuloendothelial system. Unconjugated bilirubin is a product of heme catabolism (Figure 9). Degradation of heme involves its conversion to biliverdin followed by reduction of biliverdin to bilirubin[148,149]. Two different enzyme systems are involved in the production of bilirubin from heme: (1) microsomal heme oxygenase[150-152]; and (2) cytosolic biliverdin reductase[153,154].

The heme oxygenase activity is detectable in all body tissues. However, the highest activity of this enzyme is found in the spleen[150]. Heme oxygenase has an evident stereospecificity for the α-meso-bridge of a heme molecule, resulting in the formation of primarily IX-α isomers of biliverdin in man.

Cytosolic biliverdin reductase is also present in all body tissues; its activity is most pronounced in the spleen, liver, and kidney.

A gram of hemoglobin produces 36.2 mg of bilirubin[151]. In an adult, the daily bilirubin production determined by different methods is 3.9 ± 0.7 mg/kg (or 250-350 mg/d) - when determined with labeled bilirubin; and 4.4 ± 0.7 mg/kg established from CO_2 formation when heme is converted to bilirubin[149,154]. Normally, a human being generates bilirubin IX-α and a minor quantity of its structural isomers: bilirubins IX-β, IX-γ or IX-δ.

Bilirubin is liberated from the endothelial tissue cells to plasma, binds to albumin, and is delivered to the hepatocytes and taken up by liver cells in a form dissociated from albumin. Bilirubin transport across the basolateral part of a membrane within the hepatocyte is accomplished by transport systems such as organic anions transport protein[155], *via* the flip-flop mechanism[156], or by simple diffusion due to chemical concentration gradient. The uptake of bilirubin is highly effective because of its rapid hepatic metabolism and excretion into the bile and of the presence of cytosolic binding proteins, such as glutathione-*S*-transferase. Unconjugated bilirubin is a nonpolar (fat-soluble) substance. In the endoplasmic reticulum of hepatocytes, bilirubin is converted by microsomal UDP-glucuronyl transferase to a polar conjugate with glucuronic acid. Mono- and diglucuronide bilirubin is formed. Water-soluble conjugate of bilirubin is easily excreted into bile. Much smaller fractions of bilirubin are conjugated to sulfates, glucose, or xylose[157].

The physiological significance of conjugation of bilirubin to sugars or glucuronic acid is diminishing its toxic properties through increasing molecular hydrophilicity, which improves bilirubin transport across the membrane structures and ensures secretion into the bile. However, IXβ, IXγ, and IXδ isomers of bilirubin require no conjugation for bile secretion because they have no rigid structure as compared to the molecule of bilirubin IXα. The latter has a rigid structure due to intramolecular hydrogen bonds. Interestingly, the destruction of the bonds can improve the biliary secretion of bilirubin IXα. It has been demonstrated that UV blood irradiation results in destruction of intramolecular hydrogen bonds in bilirubin. This facilitates the diffusion of bilirubin IXα molecules across the membrane structures without conjugation with glucuronic acid or sugars.

A greater proportion of conjugated bilirubin in the bile is present in the mixed micelles containing cholesterol, phospholipids, and bile acids. Conjugated bilirubin is hydrolyzed by bacterial glucuronidases under urobilinogens. Having a nonpolar molecule, urobilinogen is well absorbed in the small intestine, and in minimal quantities in the colon. The liver and kidneys re-excrete small quantities of absorbed urobilinogen. In hepatocyte dysfunction, hepatic urobilinogen re-excretion is impaired and renal excretion is increased[151,153,154].

SECRETION OF WATER AND ELECTROLYTES INTO BILE

Formation of bile and generation of bile flow are driven by the active secretion of bile salts, lipids and electrolytes into the canalicular and bile duct lumens followed by the osmotic movement of water[158]. Thus, water has to cross rapidly into and out of the cell interior driven by osmotic forces. Bile as a fluid, results from complicated interplay of hepatocyte and cholangiocyte uptake, secretion and concentration that involves various transporters of lipids, anions, cations, and water. Considering bile is composed of > 95% water, the molecular basis and regulatory mechanisms of water transport in hepatocytes during bile formation are still under evaluation[159].

Theoretically, water can flow through the hepatocyte epithelial barrier either across tight junctions between adjacent hepatocytes (paracellular route) or across hepatocyte plasma membranes (transcellular route). The paracellular route was traditionally proposed as the major pathway for water movement.

The tight junctions play an important role in the paracellular secretion of water and the dissolved low-molecular-weight compounds of the latter. Water and electrolytes are assumed to pass from the intercellular space through the tight junctions into the bile canalicular lumen[99]. Moreover, the selectivity of electrolyte excretion is considered to be cause by the presence of a negative charge at the site of a tight junction. This charge serves also as a barrier to regurgitation of substances from the bile canaliculus into the sinusoidal space. The molecular mechanisms of paracellular permeability are currently associated with the functioning of the specific tight junction protein ZO-1. ZO-1 is phosphorylated with protein kinases, which is of importance in the molecular mechanisms responsible for the regulation of paracellular permeability[160]. Tight junctions exhibit low water permeability but allow electrolyte permeation that enables canalicular spaces to shrink below the van't Hoff equilibrium during the osmotic maneuver[161]. Nonetheless, the experimental data supporting this view remain limited and largely indirect[162].

The cloning and functional characterization of a family of proteins that works as membrane water channels, named AQPs[163], challenged the former concepts of water transport and contributed to the better understanding of bile physiology. The discovery of the AQP water channels has clarified the mechanisms by which water, the major component of bile, moves across the hepatobiliary epithelia[164].

Direct osmotic water permeability assessment by stopped-flow spectrophotometry in canalicular and sinusoidal plasma membrane vesicles revealed the presence of both lipid (non-channel) and AQP-mediated pathways for sinusoidal and canalicular water movement[165]. The study demonstrated that the canalicular plasma membrane domain has lower water permeability than the sinusoidal membrane, and thus it is rate limiting for transcellular water transport in hepatocytes[159]. However, upon cAMP stimulus the intracellular AQP8 inserts to the canalicular domain and so this membrane becomes highly water permeable. Therefore, the transcellular pathway *via* water channels seems to account for most of the water entering the bile canaliculus. Hepatocytes express AQPs, a family of membrane channel proteins that facilitate the osmotically driven movement of water molecules. AQP8 is localized to canalicular membranes and modulates membrane water permeability, providing a molecular mechanism for the osmotically coupled transport of solute and water during bile formation.

There is experimental evidence suggesting that defective hepatocyte AQP8 expression leads to alterations in normal bile physiology. Thus, AQP8 protein is down-regulated (and canalicular water permeability decreased), in established rat models of cholestasis, such as sepsis-associated cholestasis, estrogen-induced cholestasis, and extrahepatic obstructive cholestasis[166].

The entry of inorganic cations and anions into the hepatocytes is effected with the participation of specific ion channels and carriers, which include Na^+,K^+-ATPase, Na^+/H^+-exchanger, Na^+/HCO_3^- cotransporter, and Na^+-independent carrier of SO_4^{2-}[153]. It is suggested[167] that by analogy with the mechanisms of water reabsorption from the bile in the gallbladder, water is reabsorbed by the bile duct epithelial cells as a result of Na^+-coupled cotransport of Cl^-. Enhanced reabsorption after removal of the gallbladder is regarded as an adaptive mechanism that provides bile concentration.

The primary mechanism of biliary copper excretion involves ATP7B-mediated vesicular sequestration of copper rather than direct copper translocation across the canalicular membrane[168].

The microfilament system is of no small importance in the canalicular secretion of bile. Bile is transported to the ducts *via* contraction of a canaliculus, which is associated with the activity of microfilaments. The latter play a great role in the regulation of permeability of a tight junction for water and electrolytes.

SECRETION OF PROTEINS INTO BILE

Proteins, as bile acids, are referred to as the osmotically active components of bile, which determine the rate of its secretion[99]. Albumin and the other proteins that are close to its molecular weight make a major contribution to the creation of an osmotic gradient.

Tens of different proteins with a molecular weight of 6-220 kDa are detectable in the bile[99]. The greater part of them are blood proteins; the lesser are the proteins entering the bile directly from the hepatocytes and epithelial cells of bile ducts. The experimental data[99] suggest that many plasma proteins interact with the specific receptors available on the sinusoidal membrane of hepatocytes and form a plasma protein-receptor complex that enters the liver cells *via* endocytosis.

The intracellular transfer of proteins from the perisinusoidal area of a hepatocyte to its pericanalicular one is mainly accomplished by targeted vesicular transport. The large proportion of proteins entering the hepatocyte from blood as a receptor-plasma protein complex generate a receptosome (endosome) and are transported in such a form to the Golgi apparatus, then to the canalicular membrane[99]. The newly synthesized proteins entering the bile canaliculi are also transported just from the hepatocytes by targeted vesicular transport. It is presumed that apolipoproteins perform in the bile the same function as lipoproteins in the serum, that is, ensure lipid transport[169].

Apoproteins are taken up from the sinusoidal space by endocytosis, transported in the hepatocytes as vesicles and released into the bile across the canalicular membrane by exocytosis[170].

In conclusion, the cholesterol that is newly synthesized in the hepatocytes serves as a substrate for the synthesis of bile acids. The synthesis of bile acids is a key point in the formation of bile. Acid-dependent and acid-independent bile fractions are identified. The structure of a hepatocyte provides a targeted transport of bile constituents from the basolateral to apical membrane of a liver cell. The transport of bile acids across the sinusoidal membrane is associated with the overcoming of a high concentration gradient and an electrical potential and it is accomplished by an active transport with the participation of carrier proteins. The vesicles are the basic form of the transport of lipids in and out of the cell itself. Bile lipids are secreted into the bile canaliculi as monolamellar vesicles. Bile acids pass across the apical part of a hepatocytic membrane independently, by ABC transporters, solubilize the phospholipids and cholesterol from the membrane surface in the bile canalicular lumen.

There is a constant transformation of vesicles to and from micelles in the bile canaliculi and ducts as a result of absorptive and secretory processes with the participation of bile acids. All the aforesaid suggests considerable progress made in the understanding of the mechanisms of bile formation and excretion, which has made possible due to the advances of fundamental investigations in cell biology, molecular biology and biochemistry.

ACKNOWLEDGMENTS

The author to express thanks to professor Gennadiy Vasilievich Saparin and Peter Valentinovich Ivannikov for receiving bile image by CCL SEM. The author expresses his gratitude to professor Vladimir Mitrofanovich Pisarev for assistance and advice in preparing the article.

REFERENCES

1 **Crawford JM**, Möckel GM, Crawford AR, Hagen SJ, Hatch VC, Barnes S, Godleski JJ, Carey MC. Imaging biliary lipid secretion in the rat: ultrastructural evidence for vesiculation of the hepatocyte canalicular membrane. *J Lipid Res* 1995; **36**: 2147-2163 [PMID: 8576641]

2 **Esteller A**. Physiology of bile secretion. *World J Gastroenterol* 2008; **14**: 5641-5649 [PMID: 18837079 DOI: 10.3748/wjg.14.5641]

3 **Poupon R**. [Molecular mechanisms of bile formation and cholestatic diseases]. *Bull Acad Natl Med* 2003; **187**: 1261-1274; discussion 1261-1724 [PMID: 15146603]

4 **Pavlov IP**. Lectures on Physiology 1912-1913. I. Physiology of digestion. In: Kupalov PS. Lecture 23: Procedure for obtaining bile. Value of bile in a digestive process. Razenkov IP, editor. Moscow: Izdatelstvo Akademii Meditsinskikh Nauk USSR, 1949

5 **Arrese M**, Trauner M. Molecular aspects of bile formation and cholestasis. *Trends Mol Med* 2003; **9**: 558-564 [PMID: 14659471 DOI: 10.1016/j.molmed.2003.10.002]

6 **Kubitz R**, Häussinger D. Osmoregulation of bile formation. *Methods Enzymol* 2007; **428**: 313-324 [PMID: 17875426 DOI: 10.1016/S0076-6879(07)28018-8]

7 **Alvaro D**, Cantafora A, Attili AF, Ginanni Corradini S, De Luca C, Minervini G, Di Biase A, Angelico M. Relationships between bile salts hydrophilicity and phospholipid composition in bile of various animal species. *Comp Biochem Physiol B* 1986; **83**: 551-554 [PMID: 3956167 DOI: 10.1016/0305-0491(86)90295-6]

8 **Loginov AS**, Chebanov SM, Saparin GV, Obyden SK. The morphology and composition of cholesterol, protein, and bilirubin deposits in dried human bile: cathodoluminescence and backscattered electron imaging. *Scanning* 1998; **20**: 442-446 [PMID: 9825540 DOI: 10.1002/sca.1998.4950200604]

9 **Fu D**, Lippincott-Schwartz J, Arias IM. Cellular mechanism of bile acid-accelerated hepatocyte polarity. *Small GTPases* 2011; **2**: 314-317 [PMID:22545229 DOI: 10.4161/sgtp.2.6.18087]

10 **Bryant DM**, Mostov KE. From cells to organs: building polarized tissue. *Nat Rev Mol Cell Biol* 2008; **9**: 887-901 [PMID: 18946477 DOI: 10.1038/nrm2523]

11 **Kojima T**, Yamamoto T, Murata M, Chiba H, Kokai Y, Sawada N. Regulation of the blood-biliary barrier: interaction between gap and tight junctions in hepatocytes. *Med Electron Microsc* 2003; **36**: 157-164 [PMID: 14505059 DOI: 10.1007/s00795-003-0220-5]

12 **Meier PJ**. Transport polarity of hepatocytes. *Semin Liver Dis* 1988; **8**: 293-307 [PMID: 2463674 DOI: 10.1055/s-2008-1040551]

13 **Ballatori N**, Clarkson TW. Biliary secretion of glutathione and of glutathione-metal complexes. *Fundam Appl Toxicol* 1985; **5**: 816-831 [PMID: 4065458 DOI: 10.1016/0272-0590(85)90165-4]

14 **Keppler D**. Export pumps for glutathione S-conjugates. *Free Radiac Biol Med* 1999; **27**: 985-991 [PMID:10569630 DOI: 10.1016/S0891-5849(99)00171-9]

15 **Tsukada N**, Ackerley CA, Phillips MJ. The structure and organization of the bile canalicular cytoskeleton with special reference to actin and actin-binding proteins. *Hepatology* 1995; **21**: 1106-1113 [PMID: 7705786]

16 **Vasudevan DM**, Sreekumari S, Vaidyanathan K. Textbook of Biochemistry for Medical Students. Chapter 13. Cholesterol and lipoproteins. 7th ed. New Delhi: Jaypee brothers medical publishers (P) Ltd, 2013: 169-183

17 **Partington JR**. A History of Chemistry, Vol. IV. London: Macmillan and Co.,Ltd., 1964: 335

18 **Marry R**, Grenner D, Mayes P, Roduell V. Human biochemistry. Moscow: Mir Publishers, 1993: 281-284

19 **Nilsson LM**, Sjövall J, Strom S, Bodin K, Nowak G, Einarsson C, Ellis E. Ethanol stimulates bile acid formation in primary human hepatocytes. *Biochem Biophys Res Commun* 2007; **364**: 743-747 [PMID: 17976534 DOI: 10.1016/j.bbrc.2007.10.039]

20 **Chiang JY**. Bile acids: regulation of synthesis. *J Lipid Res* 2009; **50**: 1955-1966 [PMID: 19346330 DOI: 10.1194/jlr.R900010-JLR200]

21 **Smith LP**, Nierstenhoefer M, Yoo SW, Penzias AS, Tobiasch E, Usheva A. The Bile Acid Synthesis Pathway Is Present and Functional in the Human Ovary. *PLoS One* 2009; 4: e7333 [PMID:19806215 DOI: 10.1371/journal.pone.0007333]

22 **Ferdinandusse S**, Houten SM. Peroxisomes and bile acid biosynthesis. *Biochim Biophys Acta* 2006; **1763**: 1427-1440 [PMID: 17034878 DOI: 10.1016/j.bbamcr.2006.09.001]

23 **Russell DW**. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; **72**: 137-174 [PMID:12543708]

24 **Carey MC**. Pathogenesis of gallstones. *Am J Surg* 1993; **165**: 410-419 [PMID: 8480873 DOI: 10.1016/S0002-9610(05)80932-8]

25 **Li T**, Jahan A, Chiang JY. Bile acids and cytokines inhibit the human cholesterol 7 alpha-hydroxylase gene via the JNK/c-jun pathway in human liver cells. *Hepatology* 2006; **43**: 1202-1210 [PMID: 16729332 DOI: 10.1002/hep.21183]

26 **Li T**, Kong X, Owsley E, Ellis E, Strom S, Chiang JY. Insulin regulation of cholesterol 7alpha-hydroxylase expression in human hepatocytes: roles of forkhead box O1 and sterol regulatory element-binding protein 1c. *J Biol Chem* 2006; **281**: 28745-28754 [PMID: 16885156 DOI: 10.1074/jbc.M605815200]

27 **Song KH**, Chiang JY. Glucagon and cAMP inhibit cholesterol 7alpha-hydroxylase (CYP7A1) gene expression in human hepatocytes: discordant regulation of bile acid synthesis and gluconeogenesis. *Hepatology* 2006; **43**: 117-125 [PMID: 16323215 DOI: 10.1002/hep.20919]

28 **Song KH**, Ellis E, Strom S, Chiang JY. Hepatocyte growth factor signaling pathway inhibits cholesterol 7alpha-hydroxylase and bile acid synthesis in human hepatocytes. *Hepatology* 2007; **46**: 1993-2002 [PMID: 17924446 DOI: 10.1002/hep.21878]

29 **Song KH**, Li T, Owsley E, Strom S, Chiang JY. Bile acids activate fibroblast growth factor 19 signaling in human hepatocytes to inhibit cholesterol 7alpha-hydroxylase gene expression. *Hepatology* 2009; **49**: 297-305 [PMID: 19085950 DOI: 10.1002/hep.22627]

30 **Bove KE**, Heubi JE, Balistreri WF, Setchell KD. Bile acid synthetic defects and liver disease: a comprehensive review. *Pediatr Dev Pathol* 2004; **7**: 315-334 [PMID: 15383928 DOI: 10.1007/s10024-002-1201-8]

31 **Chiang JY**. Bile acid regulation of gene expression: roles of nuclear hormone receptors. *Endocr Rev* 2002; **23**: 443-463 [PMID: 12202460 DOI: 10.1210/er.2000-0035]

32 **Chiang JY**. Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. *J Hepatol* 2004; **40**: 539-551 [PMID: 15123373 DOI: 10.1016/j.jhep.2003.11.006]

33 **Chiang JY**, Kimmel R, Weinberger C, Stroup D. Farnesoid X receptor responds to bile acids and represses cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription. *J Biol Chem* 2000; **275**: 10918-10924 [PMID: 10753890 DOI: 10.1074/jbc.275.15.10918]

34 **Yang Y**, Zhang M, Eggertsen G, Chiang JY. On the mechanism of bile acid inhibition of rat sterol 12alpha-hydroxylase gene (CYP8B1) transcription: roles of alpha-fetoprotein transcription factor and hepatocyte nuclear factor 4alpha. *Biochim Biophys Acta* 2002; **1583**: 63-73 [PMID: 12069850 DOI: 10.1016/S1388-1981(02)00186-5]

35 **Chen W**, Chiang JY. Regulation of human sterol 27-hydroxylase gene (CYP27A1) by bile acids and hepatocyte nuclear factor 4alpha (HNF4alpha). *Gene* 2003; **313**: 71-82 [PMID: 12957378 DOI: 10.1016/S0378-1119(03)00631-0]

36 **Hunt MC**, Yang YZ, Eggertsen G, Carneheim CM, Gåfvels M, Einarsson C, Alexson SE. The peroxisome proliferator-activated receptor alpha (PPARalpha) regulates bile acid biosynthesis. *J Biol Chem* 2000; **275**: 28947-28953 [PMID: 10867000 DOI: 10.1074/jbc.M002782200]

37 **Pineda Torra I**, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B. Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. *Mol Endocrinol* 2003; **17**: 259-272 [PMID: 12554753 DOI: 10.1210/me.2002-0120]

38 **Shefer S**, Salen G, Nguyen L, Batta AK, Packin V, Tint GS, Hauser S. Competitive inhibition of bile acid synthesis by endogenous cholestanol and sitosterol in sitosterolemia with xanthomatosis. Effect on cholesterol 7 alpha-hydroxylase. *J Clin Invest* 1988; **82**: 1833-1839 [PMID: 3143743 DOI: 10.1172/JCI113799]

39 **Gälman C**, Angelin B, Rudling M. Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. *Gastroenterology* 2005; **129**: 1445-1453 [PMID: 16285946 DOI: 10.1053/j.gastro.2005.09.009]

40 **Dietschy JM**, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J Lipid Res* 1993; **34**: 1637-1659 [PMID: 8245716]

41 **Dietschy JM**, Turley SD. Control of cholesterol turnover in the mouse. *J Biol Chem* 2002; **277**: 3801-3804 [PMID: 11733542 DOI: 10.1074/jbc.R100057200]

42 **Small DM**, Rapo S. Source of abnormal bile in patients with cholesterol gallstones. *N Engl J Med* 1970; **283**: 53-57 [PMID: 5420358 DOI: 10.1056/NEJM197007092830201]

43 **Gu JJ**, Hofmann AF, Ton-Nu HT, Schteingart CD, Mysels KJ. Solubility of calcium salts of unconjugated and conjugated natural bile acids. *J Lipid Res* 1992; **33**: 635-646 [PMID:

1619359]
44 **Marcinkiewicz J**, Kontny E. Taurine and inflammatory diseases. *Amino Acids* 2012; Epub ahead of print [PMID: 22810731]
45 **Chen W**, Guo JX, Chang P. The effect of taurine on cholesterol metabolism. *Mol Nutr Food Res* 2012; **56**: 681-690 [PMID: 22648615 DOI: 10.1002/mnfr.201100799]
46 **Inoue Y**, Yu AM, Inoue J, Gonzalez FJ. Hepatocyte nuclear factor 4alpha is a central regulator of bile acid conjugation. *J Biol Chem* 2004; **279**: 2480-2489 [PMID: 14583614 DOI: 10.1074/jbc.M311015200]
47 **Jansen PL**, Mulder GJ, Burchell B, Bock KW. New developments in glucuronidation research: report of a workshop on "glucuronidation, its role in health and disease". *Hepatology* 1992; **15**: 532-544 [PMID: 1531971 DOI: 10.1002/hep.1840150328]
48 **Stiehl A**. Disturbances of bile acid metabolism in cholestasis. *Clin Gastroenterol* 1977; **6**: 45-67 [PMID: 330052]
49 **Yousef I**, Mignault D, Tuchweber B. Effect of complete sulfation of bile acids on bile formation: role of conjugation and number of sulfate groups. *Hepatology* 1992; **15**: 438-445 [PMID: 1544624 DOI: 10.1002/hep.1840150314]
50 **Hofmann AF**. Detoxification of lithocholic acid, a toxic bile acid: relevance to drug hepatotoxicity. *Drug Metab Rev* 2004; **36**: 703-722 [PMID: 15554243 DOI: 10.1081/DMR-200033475]
51 **Hofmann AF**, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell Mol Life Sci* 2008; **65**: 2461-2483 [PMID: 18488143 DOI: 10.1007/s00018-008-7568-6]
52 **Monte MJ**, Marin JJ, Antelo A, Vazquez-Tato J. Bile acids: chemistry, physiology, and pathophysiology. *World J Gastroenterol* 2009; **15**: 804-816 [PMID: 19230041 DOI: 10.3748/wjg.15.804]
53 **Kawamoto T**, Okano G, Akino T. Biosynthesis and turnover of individual molecular species of phosphatidylcholine in liver and bile. *Biochim Biophys Acta* 1980; **619**: 20-34 [PMID: 7417466 DOI: 10.1016/0005-2760(80)90239-8]
54 **Carey MC**, Lamont JT. Cholesterol gallstone formation. 1. Physical-chemistry of bile and biliary lipid secretion. *Prog Liver Dis* 1992; **10**: 139-163 [PMID: 1296228]
55 **Ivanchenkova RA**. Neurohumoral regulation of bile production and excretion processes. *Klinicheskaya Meditsina* 1986; **64**: 24-32
56 **Nilsson S**, Scherstén T. Importance of bile acids for phospholipid secretion into human hepatic bile. *Gastroenterology* 1969; **57**: 525-532 [PMID: 5348087]
57 **Groen AK**, Oude Elferink RP. Lipid transport into bile and role in bile formation. *Curr Drug Targets Immune Endocr Metabol Disord* 2005; **5**: 131-135 [PMID: 16089344 DOI: 10.2174/1568008054064887]
58 **Crawford AR**, Smith AJ, Hatch VC, Oude Elferink RP, Borst P, Crawford JM. Hepatic secretion of phospholipid vesicles in the mouse critically depends on mdr2 or MDR3 P-glycoprotein expression. Visualization by electron microscopy. *J Clin Invest* 1997; **100**: 2562-2567 [PMID: 9366571 DOI: 10.1172/JCI119799]
59 **Patton GM**, Fasulo JM, Robins SJ. Hepatic phosphatidylcholines: evidence for synthesis in the rat by extensive reutilization of endogenous acylglycerides. *J Lipid Res* 1994; **35**: 1211-1221 [PMID: 7964182]
60 **Cohen DE**, Leonard MR, Carey MC. In vitro evidence that phospholipid secretion into bile may be coordinated intracellularly by the combined actions of bile salts and the specific phosphatidylcholine transfer protein of liver. *Biochemistry* 1994; **33**: 9975-9980 [PMID: 8061007 DOI: 10.1021/bi00199a021]
61 **Berr F**, Meier PJ, Stieger B. Evidence for the presence of a phosphatidylcholine translocator in isolated rat liver canalicular plasma membrane vesicles. *J Biol Chem* 1993; **268**: 3976-3979 [PMID: 8440689]
62 **Crawford JM**. Role of vesicle-mediated transport pathways in hepatocellular bile secretion. *Semin Liver Dis* 1996; **16**: 169-189 [PMID: 8781022 DOI: 10.1055/s-2007-1007230]
63 **Elferink RP**, Tytgat GN, Groen AK. Hepatic canalicular membrane 1: The role of mdr2 P-glycoprotein in hepatobiliary lipid transport. *FASEB J* 1997; **11**: 19-28 [PMID: 9034162]
64 **van de Heijning BJ**, Stolk MF, van Erpecum KJ, Renooij W, Groen AK, vanBerge-Henegouwen GP. Bile salt-induced cholesterol crystal formation from model bile vesicles: a time course study. *J Lipid Res* 1994; **35**: 1002-1011 [PMID: 8077840]
65 **Carey MC**, Cahalane MJ. Whither biliary sludge? *Gastroenterology* 1988; **95**: 508-523 [PMID: 3292341]
66 **Möckel GM**, Gorti S, Tandon RK, Tanaka T, Carey MC. Microscope laser light-scattering spectroscopy of vesicles within canaliculi of rat hepatocyte couplets. *Am J Physiol* 1995; **269**: G73-G84 [PMID: 7631803]
67 **Paumgartner G**, Sauerbruch T. Secretion, composition and flow of bile. *Clin Gastroenterol* 1983; **12**: 3-23 [PMID: 6347458]
68 **Sperber I**. Secretion of organic anions in the formation of urine and bile. *Pharmacol Rev* 1959; **11**: 109-134 [PMID: 13633440]
69 **Gumucio JJ**, Miller DL. Functional implications of liver cell heterogeneity. *Gastroenterology* 1981; **80**: 393-403 [PMID: 7004997]
70 **Anwer MS**, Hegner D. Role of inorganic electrolytes in bile acid-independent canalicular bile formation. *Am J Physiol* 1983; **244**: G116-G124 [PMID: 6130705]
71 **Dawson PA**, Lan T, Rao A. Bile acid transporters. *J Lipid Res* 2009; **50**: 2340-2357 [PMID: 19498215 DOI: 10.1194/jlr.R900012-JLR2001]
72 **Krag E**, Phillips SF. Active and passive bile acid absorption in man. Perfusion studies of the ileum and jejunum. *J Clin Invest* 1974; **53**: 1686-1694 [PMID: 4830231 DOI: 10.1172/JCI107720]
73 **Schiff ER**, Small NC, Dietschy JM. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. *J Clin Invest* 1972; **51**: 1351-1362 [PMID: 5024036 DOI: 10.1172/JCI106931]
74 **Dawson PA**, Hubbert M, Haywood J, Craddock AL, Zerangue N, Christian WV, Ballatori N. The heteromeric organic solute transporter alpha-beta, Ostalpha-Ostbeta, is an ileal basolateral bile acid transporter. *J Biol Chem* 2005; **280**: 6960-6968 [PMID: 15563450 DOI: 10.1074/jbc.M412752200]
75 **Ballatori N**, Christian WV, Lee JY, Dawson PA, Soroka CJ, Boyer JL, Madejczyk MS, Li N. OSTalpha-OSTbeta: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. *Hepatology* 2005; **42**: 1270-1279 [PMID: 16317684 DOI: 10.1002/hep.20961]
76 **Hofmann AF**, Schteingart CD, Lillienau J. Biological and medical aspects of active ileal transport of bile acids. *Ann Med* 1991; **23**: 169-175 [PMID: 2069793 DOI: 10.3109/07853899109148043]
77 **Denson LA**, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, Karpen SJ. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* 2001; **121**: 140-147 [PMID: 11438503 DOI: 10.1053/gast.2001.25503]
78 **Meier PJ**. The bile salt secretory polarity of hepatocytes. *J Hepatol* 1989; **9**: 124-129 [PMID: 2671118 DOI: 10.1016/0168-8278(89)90085-8]
79 **Shaffer EA**. Cholestasis: the ABCs of cellular mechanisms for impaired bile secretion--transporters and genes. *Can J Gastroenterol* 2002; **16**: 380-389 [PMID: 12096302]
80 **Alrefai WA**, Gill RK. Bile acid transporters: structure, function, regulation and pathophysiological implications. *Pharm Res* 2007; **24**: 1803-1823 [PMID: 17404808 DOI: 10.1007/s11095-007-9289-1]
81 **Trauner M**, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev* 2003; **83**: 633-671 [PMID: 12663868]

82 **Lam P**, Soroka CJ, Boyer JL. The bile salt export pump: clinical and experimental aspects of genetic and acquired cholestatic liver disease. *Semin Liver Dis* 2010; **30**: 125-133 [PMID: 20422495 DOI: 10.1055/s-0030-1253222]

83 **Roma MG**, Crocenzi FA, Mottino AD. Dynamic localization of hepatocellular transporters in health and disease. *World J Gastroenterol* 2008; **14**: 6786-6801 [PMID: 19058304 DOI: 10.3748/wjg.14.6786]

84 **Reichen J**. The Role of the Sinusoidal Endothelium in Liver Function. *News Physiol Sci* 1999; **14**: 117-121 [PMID: 11390834]

85 **Frimmer M**, Ziegler K. The transport of bile acids in liver cells. *Biochim Biophys Acta* 1988; **947**: 75-99 [DOI: 10.1016/0304-4157(88)90020-2]

86 **Hagenbuch B**, Meier PJ. Sinusoidal (basolateral) bile salt uptake systems of hepatocytes. *Semin Liver Dis* 1996; **16**: 129-136 [PMID: 8781018 DOI: 10.1055/s-2007-1007226]

87 **Hofmann AF**. Biliary secretion and excretion in health and disease: current concepts. *Ann Hepatol* 2007; **6**: 15-27 [PMID: 17297425]

88 **Erlinger S**. Bile flow. In: Arias IM, Popper H, Jacoby WB, Schachter D, Shafritz DA, editors. The Liver: Biology and Pathobiology. New York: Raven Press, 1988: 643-664

89 **Meier PJ**, Eckhardt U, Schroeder A, Hagenbuch B, Stieger B. Substrate specificity of sinusoidal bile acid and organic anion uptake systems in rat and human liver. *Hepatology* 1997; **26**: 1667-1677 [PMID: 9398014 DOI: 10.1002/hep.510260641]

90 **Meier PJ**, Stieger B. Bile salt transporters. *Annu Rev Physiol* 2002; **64**: 635-661 [PMID: 11826283 DOI: 10.1146/annurev.physiol.64.082201.100300]

91 **Arrese M**, Ananthananarayanan M, Suchy FJ. Hepatobiliary transport: molecular mechanisms of development and cholestasis. *Pediatr Res* 1998; **44**: 141-147 [PMID: 9702905 DOI: 10.1203/00006450-199808000-00001]

92 **Bohan A**, Boyer JL. Mechanisms of hepatic transport of drugs: implications for cholestatic drug reactions. *Semin Liver Dis* 2002; **22**: 123-136 [PMID: 12016544 DOI: 10.1055/s-2002-30099]

93 **Hagenbuch B**, Meier PJ. Molecular cloning, chromosomal localization, and functional characterization of a human liver Na+/bile acid cotransporter. *J Clin Invest* 1994; **93**: 1326-1331 [PMID: 8132774 DOI: 10.1172/JCI117091]

94 **Hagenbuch B**, Dawson P. The sodium bile salt cotransport family SLC10. *Pflugers Arch* 2004; **447**: 566-570 [PMID: 12851823 DOI: 10.1007/s00424-003-1130-z]

95 **Geyer J**, Wilke T, Petzinger E. The solute carrier family SLC10: more than a family of bile acid transporters regarding function and phylogenetic relationships. *Naunyn Schmiedebergs Arch Pharmacol* 2006; **372**: 413-431 [PMID: 16541252 DOI: 10.1007/s00210-006-0043-8]

96 **Godoy JR**, Fernandes C, Döring B, Beuerlein K, Petzinger E, Geyer J. Molecular and phylogenetic characterization of a novel putative membrane transporter (SLC10A7), conserved in vertebrates and bacteria. *Eur J Cell Biol* 2007; **86**: 445-460 [PMID: 17628207 DOI: 10.1016/j.ejcb.2007.06.001]

97 **Blitzer BL**, Boyer JL. Cytochemical localization of Na+, K+-ATPase in the rat hepatocyte. *J Clin Invest* 1978; **62**: 1104-1108 [PMID: 213446 DOI: 10.1172/JCI109216]

98 **Latham PS**, Kashgarian M. The ultrastructural localization of transport ATPase in the rat liver at non-bile canalicular plasma membranes. *Gastroenterology* 1979; **76**: 988-996 [PMID: 220132]

99 **Blitzer BL**, Boyer JL. Cellular mechanisms of bile formation. *Gastroenterology* 1982; **82**: 346-357 [PMID: 6274732]

100 **Sztul ES**, Biemesderfer D, Caplan MJ, Kashgarian M, Boyer JL. Localization of Na+,K+-ATPase alpha-subunit to the sinusoidal and lateral but not canalicular membranes of rat hepatocytes. *J Cell Biol* 1987; **104**: 1239-1248 [PMID: 3032985 DOI: 10.1083/jcb.104.5.1239]

101 **Boyer JL**, Ng OC, Ananthanarayanan M, Hofmann AF, Schteingart CD, Hagenbuch B, Stieger B, Meier PJ. Expression and characterization of a functional rat liver Na+ bile acid cotransport system in COS-7 cells. *Am J Physiol* 1994; **266**: G382-G387 [PMID: 8166278]

102 **Hata S**, Wang P, Eftychiou N, Ananthanarayanan M, Batta A, Salen G, Pang KS, Wolkoff AW. Substrate specificities of rat oatp1 and ntcp: implications for hepatic organic anion uptake. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G829-G839 [PMID: 12842829]

103 **Kramer W**, Stengelin S, Baringhaus KH, Enhsen A, Heuer H, Becker W, Corsiero D, Girbig F, Noll R, Weyland C. Substrate specificity of the ileal and the hepatic Na(+)/bile acid cotransporters of the rabbit. I. Transport studies with membrane vesicles and cell lines expressing the cloned transporters. *J Lipid Res* 1999; **40**: 1604-1617 [PMID: 10484607]

104 **Platte HD**, Honscha W, Schuh K, Petzinger E. Functional characterization of the hepatic sodium-dependent taurocholate transporter stably transfected into an immortalized liver-derived cell line and V79 fibroblasts. *Eur J Cell Biol* 1996; **70**: 54-60 [PMID: 8738419]

105 **Mita S**, Suzuki H, Akita H, Stieger B, Meier PJ, Hofmann AF, Sugiyama Y. Vectorial transport of bile salts across MDCK cells expressing both rat Na+-taurocholate cotransporting polypeptide and rat bile salt export pump. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G159-G167 [PMID: 15297262 DOI: 10.1152/ajpgi.00360.2003]

106 **Mita S**, Suzuki H, Akita H, Hayashi H, Onuki R, Hofmann AF, Sugiyama Y. Vectorial transport of unconjugated and conjugated bile salts by monolayers of LLC-PK1 cells doubly transfected with human NTCP and BSEP or with rat Ntcp and Bsep. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G550-G556 [PMID: 16474011 DOI: 10.1152/ajpgi.00364.2005]

107 **Craddock AL**, Love MW, Daniel RW, Kirby LC, Walters HC, Wong MH, Dawson PA. Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter. *Am J Physiol* 1998; **274**: G157-G169 [PMID: 9458785]

108 **Pellicoro A**, Faber KN. Review article: The function and regulation of proteins involved in bile salt biosynthesis and transport. *Aliment Pharmacol Ther* 2007; **26** Suppl 2: 149-160 [PMID: 18081658 DOI: 10.1111/j.1365-2036.2007.03522.x]

109 **Kullak-Ublick GA**, Hagenbuch B, Stieger B, Schteingart CD, Hofmann AF, Wolkoff AW, Meier PJ. Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* 1995; **109**: 1274-1282 [PMID: 7557095 DOI: 10.1016/0016-5085(95)90588-X]

110 **Meng LJ**, Wang P, Wolkoff AW, Kim RB, Tirona RG, Hofmann AF, Pang KS. Transport of the sulfated, amidated bile acid, sulfolithocholyltaurine, into rat hepatocytes is mediated by Oatp1 and Oatp2. *Hepatology* 2002; **35**: 1031-1040 [PMID: 11981753 DOI: 10.1053/jhep.2002.32667]

111 **Ballatori N**. Biology of a novel organic solute and steroid transporter, OSTalpha-OSTbeta. *Exp Biol Med (Maywood)* 2005; **230**: 689-698 [PMID: 16246895]

112 **van Montfoort JE**, Müller M, Groothuis GM, Meijer DK, Koepsell H, Meier PJ. Comparison of "type I" and "type II" organic cation transport by organic cation transporters and organic anion-transporting polypeptides. *J Pharmacol Exp Ther* 2001; **298**: 110-115 [PMID: 11408531]

113 **Müller M**, Jansen PL. Molecular aspects of hepatobiliary transport. *Am J Physiol* 1997; **272**: G1285-G1303 [PMID: 9227463]

114 **Ito K**, Suzuki H, Horie T, Sugiyama Y. Apical/basolateral surface expression of drug transporters and its role in vectorial drug transport. *Pharm Res* 2005; **22**: 1559-1577 [PMID: 16180115 DOI: 10.1007/s11095-005-6810-2]

115 **Niemi M**. Role of OATP transporters in the disposition of drugs. *Pharmacogenomics* 2007; **8**: 787-802 [PMID: 18240907 DOI: 10.2217/14622416.8.7.787]

116 **Kaplowitz N**. Physiological significance of glutathione S-transferases. *Am J Physiol* 1980; **239**: G439-G444 [PMID: 7446736]

117 **Crawford JM**, Berken CA, Gollan JL. Role of the hepatocyte microtubular system in the excretion of bile salts and biliary lipid: implications for intracellular vesicular transport. *J Lipid Res* 1988; **29**: 144-156 [PMID: 3367084]

118 **Lamri Y**, Roda A, Dumont M, Feldmann G, Erlinger S. Immunoperoxidase localization of bile salts in rat liver cells. Evidence for a role of the Golgi apparatus in bile salt transport. *J Clin Invest* 1988; **82**: 1173-1182 [PMID: 2459158 DOI: 10.1172/JCI113714]

119 **Crawford JM**, Vinter DW, Gollan JL. Taurocholate induces pericanalicular localization of C6-NBD-ceramide in isolated hepatocyte couplets. *Am J Physiol* 1991; **260**: G119-G132 [PMID: 1987800]

120 **Dubin M**, Maurice M, Feldmann G, Erlinger S. Influence of colchicine and phalloidin on bile secretion and hepatic ultrastructure in the rat. Possible interaction between microtubules and microfilaments. *Gastroenterology* 1980; **79**: 646-654 [PMID: 7190948]

121 **Trauner M**, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. *N Engl J Med* 1998; **339**: 1217-1227 [PMID: 9780343 DOI: 10.1056/NEJM199810223391707]

122 **Gerloff T**, Stieger B, Hagenbuch B, Madon J, Landmann L, Roth J, Hofmann AF, Meier PJ. The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. *J Biol Chem* 1998; **273**: 10046-10050 [PMID: 9545351 DOI: 10.1074/jbc.273.16.10046]

123 **Kipp H**, Pichetshote N, Arias IM. Transporters on demand: intrahepatic pools of canalicular ATP binding cassette transporters in rat liver. *J Biol Chem* 2001; **276**: 7218-7224 [PMID: 11113123 DOI: 10.1074/jbc.M007794200]

124 **Gatmaitan ZC**, Nies AT, Arias IM. Regulation and translocation of ATP-dependent apical membrane proteins in rat liver. *Am J Physiol* 1997; **272**: G1041-G1049 [PMID: 9176212]

125 **Strautnieks SS**, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, Sokal E, Dahan K, Childs S, Ling V, Tanner MS, Kagalwalla AF, Németh A, Pawlowska J, Baker A, Mieli-Vergani G, Freimer NB, Gardiner RM, Thompson RJ. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. *Nat Genet* 1998; **20**: 233-238 [PMID: 9806540 DOI: 10.1038/3034]

126 **Wakabayashi Y**, Lippincott-Schwartz J, Arias IM. Intracellular trafficking of bile salt export pump (ABCB11) in polarized hepatic cells: constitutive cycling between the canalicular membrane and rab11-positive endosomes. *Mol Biol Cell* 2004; **15**: 3485-3496 [PMID: 15121884 DOI: 10.1091/mbc.E03-10-0737]

127 **Lapierre LA**, Kumar R, Hales CM, Navarre J, Bhartur SG, Burnette JO, Provance DW, Mercer JA, Bähler M, Goldenring JR. Myosin vb is associated with plasma membrane recycling systems. *Mol Biol Cell* 2001; **12**: 1843-1857 [PMID: 11408590 DOI: 10.1091/mbc.12.6.1843]

128 **Paulusma CC**, Bosma PJ, Zaman GJ, Bakker CT, Otter M, Scheffer GL, Scheper RJ, Borst P, Oude Elferink RP. Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science* 1996; **271**: 1126-1128 [PMID: 8599091 DOI: 10.1126/science.271.5252.1126]

129 **Silverman JA**, Raunio H, Gant TW, Thorgeirsson SS. Cloning and characterization of a member of the rat multidrug resistance (mdr) gene family. *Gene* 1991; **106**: 229-236 [PMID: 1682220 DOI: 10.1016/0378-1119(91)90203-N]

130 **Suchy FJ**, Ananthanarayanan M. Bile salt excretory pump: biology and pathobiology. *J Pediatr Gastroenterol Nutr* 2006; **43** Suppl 1: S10-S16 [PMID: 16819395 DOI: 10.1097/01.mpg.0000226385.71859.5f]

131 **Kullak-Ublick GA**, Stieger B, Hagenbuch B, Meier PJ. Hepatic transport of bile salts. *Semin Liver Dis* 2000; **20**: 273-292 [PMID: 11076396 DOI: 10.1055/s-2000-9426]

132 **Akita H**, Suzuki H, Ito K, Kinoshita S, Sato N, Takikawa H, Sugiyama Y. Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. *Biochim Biophys Acta* 2001; **1511**: 7-16 [PMID: 11248200 DOI: 10.1016/S0005-2736(00)00355-2]

133 **Paulusma CC**, van Geer MA, Evers R, Heijn M, Ottenhoff R, Borst P, Oude Elferink RP. Canalicular multispecific organic anion transporter/multidrug resistance protein 2 mediates low-affinity transport of reduced glutathione. *Biochem J* 1999; **338** (Pt 2): 393-401 [PMID: 10024515 DOI: 10.1042/0264-6021:3380393]

134 **Yang B**, Hill CE. Nifedipine modulation of biliary GSH and GSSG/ conjugate efflux in normal and regenerating rat liver. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G85-G94 [PMID: 11408258]

135 **Ballatori N**, Truong AT. Glutathione as a primary osmotic driving force in hepatic bile formation. *Am J Physiol* 1992; **263**: G617-G624 [PMID: 1443136]

136 **Banales JM**, Prieto J, Medina JF. Cholangiocyte anion exchange and biliary bicarbonate excretion. *World J Gastroenterol* 2006; **12**: 3496-3511 [PMID: 16773707]

137 **Hardison WG**, Wood CA. Importance of bicarbonate in bile salt independent fraction of bile flow. *Am J Physiol* 1978; **235**: E158-E164 [PMID: 150796]

138 **Medina JF**, Lecanda J, Acín A, Ciesielczyk P, Prieto J. Tissue-specific N-terminal isoforms from overlapping alternate promoters of the human AE2 anion exchanger gene. *Biochem Biophys Res Commun* 2000; **267**: 228-235 [PMID: 10623603 DOI: 10.1006/bbrc.1999.1951]

139 **Aranda V**, Martínez I, Melero S, Lecanda J, Banales JM, Prieto J, Medina JF. Shared apical sorting of anion exchanger isoforms AE2a, AE2b1, and AE2b2 in primary hepatocytes. *Biochem Biophys Res Commun* 2004; **319**: 1040-1046 [PMID: 15184086 DOI: 10.1016/j.bbrc.2004.05.080]

140 **Marinelli RA**, Gradilone SA, Carreras FI, Calamita G, Lehmann GL. Liver aquaporins: significance in canalicular and ductal bile formation. *Ann Hepatol* 2004; **3**: 130-136 [PMID: 15657554]

141 **Huebert RC**, Splinter PL, Garcia F, Marinelli RA, LaRusso NF. Expression and localization of aquaporin water channels in rat hepatocytes. Evidence for a role in canalicular bile secretion. *J Biol Chem* 2002; **277**: 22710-22717 [PMID: 11932260 DOI: 10.1074/jbc.M202394200]

142 **García F**, Kierbel A, Larocca MC, Gradilone SA, Splinter P, LaRusso NF, Marinelli RA. The water channel aquaporin-8 is mainly intracellular in rat hepatocytes, and its plasma membrane insertion is stimulated by cyclic AMP. *J Biol Chem* 2001; **276**: 12147-12152 [PMID: 11278499 DOI: 10.1074/jbc.M009403200]

143 **Zamychkina KS**, Kriukova LV. Absorption of methionine from the gall-bladder in vivo and in vitro. *Biull Eksp Biol Med* 1967; **63**: 28-30 [PMID: 5615573]

144 **Rehman A**, Affronti J, Rao S. Sphincter of Oddi dysfunction: an evidence-based review. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 713-722 [PMID: 24161134]

145 **Roda A**, Cappelleri G, Aldini R, Roda E, Barbara L. Quantitative aspects of the interaction of bile acids with human serum albumin. *J Lipid Res* 1982; **23**: 490-495 [PMID: 7077161]

146 **Salvioli G**, Lugli R, Pradelli JM, Gigliotti G. Bile acid binding in plasma: the importance of lipoproteins. *FEBS Lett* 1985; **187**: 272-276 [PMID: 4018265 DOI: 10.1016/0014-5793(85)81257-6]

147 **Hedenborg G**, Norlander A, Norman A, Svensson A. Bile acid transport by serum lipoproteins in patients with extrahepatic cholestasis. *Scand J Clin Lab Invest* 1986; **46**: 745-750 [PMID: 3797986 DOI: 10.3109/00365518609084046]

148 **Bissell DM**. In: Bile pigments and jaundice. Ostrow JD, editor. New-York: Marcel Dekker Inc., 1986: 133-156

149 **Kapitulnik J**, Maines MD. The role of bile pigments in health and disease: effects on cell signaling, cytotoxicity, and

cytoprotection. *Front Pharmacol* 2012; **3**: 136 [PMID: 22811668 DOI: 10.3389/fphar.2012.00136]

150 **Maines MD**. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J* 1988; **2**: 2557-2568 [PMID: 3290025]

151 **Lundvig DM**, Immenschuh S, Wagener FA. Heme oxygenase, inflammation, and fibrosis: the good, the bad, and the ugly? *Front Pharmacol* 2012; **3**: 81 [PMID: 22586396 DOI: 10.3389/fphar.2012.00081]

152 **Fevery J**, Vanstapel F, Blanckaert N. Bile pigment metabolism. *Baillieres Clin Gastroenterol* 1989; **3**: 283-312 [PMID: 2655756 DOI: 10.1016/0950-3528(89)90002-X]

153 **Gibbs PE**, Tudor C, Maines MD. Biliverdin reductase: more than a namesake - the reductase, its Peptide fragments, and biliverdin regulate activity of the three classes of protein kinase C. *Front Pharmacol* 2012; **3**: 31 [PMID: 22419908 DOI: 10.3389/fphar.2012.00031]

154 **Landaw SA**, Callahan EW, Schmid R. Catabolism of heme in vivo: comparison of the simultaneous production of bilirubin and carbon monoxide. *J Clin Invest* 1970; **49**: 914-925 [PMID: 5441545 DOI: 10.1172/JCI106311]

155 **Tiribelli C**, Ostrow JD. New concepts in bilirubin chemistry, transport and metabolism: report of the Second International Bilirubin Workshop, April 9-11, 1992, Trieste, Italy. *Hepatology* 1993; **17**: 715-736 [PMID: 8477977 DOI: 10.1002/hep.1840170428]

156 **Zucker SD**, Goessling W, Gollan JL. Kinetics of bilirubin transfer between serum albumin and membrane vesicles. Insight into the mechanism of organic anion delivery to the hepatocyte plasma membrane. *J Biol Chem* 1995; **270**: 1074-1081 [PMID: 7836362 DOI: 10.1074/jbc.270.3.1074]

157 **Chowdhury JR**, Chowdhury NR. Conjugation and excretion of bilirubin. *Semin Liver Dis* 1983; **3**: 11-23 [PMID: 6403990 DOI: 10.1055/s-2008-1040667]

158 **Calamita G**, Ferri D, Gena P, Liquori GE, Marinelli RA, Meyer G, Portincasa P, Svelto M. Water transport into bile and role in bile formation. *Curr Drug Targets Immune Endocr Metabol Disord* 2005; **5**: 137-142 [PMID: 16089345 DOI: 10.2174/1568008054064850]

159 **Lehmann GL**, Larocca MC, Soria LR, Marinelli RA. Aquaporins: their role in cholestatic liver disease. *World J Gastroenterol* 2008; **14**: 7059-7067 [PMID: 19084912 DOI: 10.3748/wjg.14.7059]

160 **Fricker G**, Hugentobler G, Meier PJ, Kurz G, Boyer JL. Identification of a single sinusoidal bile salt uptake system in skate liver. *Am J Physiol* 1987; **253**: G816-G822 [PMID: 3425718]

161 **Jessner W**, Zsembery A, Graf J. Transcellular water transport in hepatobiliary secretion and role of aquaporins in liver. *Wien Med Wochenschr* 2008; **158**: 565-569 [PMID: 18998074 DOI: 10.1007/s10354-008-0597-9]

162 **Masyuk AI**, Marinelli RA, LaRusso NF. Water transport by epithelia of the digestive tract. *Gastroenterology* 2002; **122**: 545-562 [PMID: 11832467 DOI: 10.1053/gast.2002.31035]

163 **King LS**, Kozono D, Agre P. From structure to disease: the evolving tale of aquaporin biology. *Nat Rev Mol Cell Biol* 2004; **5**: 687-698 [PMID: 15340377 DOI: 10.1038/nrm1469]

164 **Portincasa P**, Calamita G. Water channel proteins in bile formation and flow in health and disease: when immiscible becomes miscible. *Mol Aspects Med* 2012; **33**: 651-664 [PMID: 22487565 DOI: 10.1016/j.mam.2012.03.010]

165 **Marinelli RA**, Tietz PS, Caride AJ, Huang BQ, LaRusso NF. Water transporting properties of hepatocyte basolateral and canalicular plasma membrane domains. *J Biol Chem* 2003; **278**: 43157-43162 [PMID: 12939275 DOI: 10.1074/jbc.M305899200]

166 **Marinelli RA**, Lehmann GL, Soria LR, Marchissio MJ. Hepatocyte aquaporins in bile formation and cholestasis. *Front Biosci (Landmark Ed)* 2011; **16**: 2642-2652 [PMID: 21622200 DOI: 10.2741/3877]

167 **Graf J**, Henderson RM, Krumpholz B, Boyer JL. Cell membrane and transepithelial voltages and resistances in isolated rat hepatocyte couplets. *J Membr Biol* 1987; **95**: 241-254 [PMID: 3585979 DOI: 10.1007/BF01869486]

168 **Cater MA**, La Fontaine S, Shield K, Deal Y, Mercer JF. ATP7B mediates vesicular sequestration of copper: insight into biliary copper excretion. *Gastroenterology* 2006; **130**: 493-506 [PMID: 16472602 DOI: 10.1053/j.gastro.2005.10.054]

169 **Ivanchenkova RA**. Some aspects of cholepoiesis. *Klinicheskaya Meditsina* 1999; **77**: 18-22

170 **Kibe A**, Holzbach RT, LaRusso NF, Mao SJ. Inhibition of cholesterol crystal formation by apolipoproteins in supersaturated model bile. *Science* 1984; **225**: 514-516 [PMID: 6429856 DOI: 10.1126/science.6429856]

P- Reviewer: Vasiliy IR **S- Editor**: Cui XM **L- Editor**: Kerr C
E- Editor: Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7361

World J Gastroenterol 2013 November 14; 19(42): 7361-7368
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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REVIEW

Role of cyclooxygenase-2 in gastric cancer development and progression

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Supported by Research Grant for Key Clinical Discipline Construction of Shanghai Municipality, China, No. ZK2012B20

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Received: July 21, 2013 **Revised:** September 17, 2013
Accepted: September 29, 2013
Published online: November 14, 2013

Abstract

Although the incidence of gastric cancer has been declining in recent decades, it remains a major public health issue as the second leading cause of cancer death worldwide. In China, gastric cancer is still the main cause of death in patients with malignant tumors. Most patients are diagnosed at an advanced stage and mortality is high. Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme in prostanoid synthesis and plays an important role in the development and progression of gastric cancer. The expression of COX-2 in gastric cancer is upregulated and its molecular mechanisms have been investigated. *Helicobacter pylori* infection, tumor suppressor gene mutation and the activation of nuclear factor-kappa B may be responsible for the elevated expression of COX-2 in gastric cancer. The mechanisms of COX-2 in the development and progression of gastric cancer are probably through promoting the proliferation of gastric cancer cells, while inhibiting apoptosis, assisting angiogenesis and lymphatic metastasis, and participating in cancer invasion and immunosuppression. This review is intended to discuss, comment and summarize recent research progress on the role of COX-2 in gastric cancer development and progression, and elucidate the molecular mechanisms which might be involved in the carcinogenesis.

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Key words: Cyclooxygenase-2; Gastric cancer; Prostaglandin; Carcinogenesis; Molecular mechanism

Core tip: Cyclooxygenase-2 (COX-2) plays an important role in gastric cancer development and progression. The present review aims to determine the molecular mechanism of COX-2 overexpression in gastric cancer and focus on the detailed information on COX-2 involved in carcinogenesis. By reviewing research progress, this may be helpful in clarifying the internal relationship of the afore-mentioned aspects.

Cheng J, Fan XM. Role of cyclooxygenase-2 in gastric cancer development and progression. *World J Gastroenterol* 2013; 19(42): 7361-7368 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7361.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7361

INTRODUCTION

Gastric cancer is one of the most common malignant tumors worldwide, and the morbidity and mortality associated with this disease are ranked second highest of all malignant neoplasms[1-3]. In China, the incidence of gastric cancer has been declining in recent years[4]. Since 75% of gastric cancer patients are diagnosed at an advanced stage and cannot be cured merely by surgery, chemotherapy combined with surgery is often the primary treatment. There are many factors that affect the prognosis of gastric cancer, and of these factors invasion and metastasis are leading causes of death. The role of cyclooxygenase-2 (COX-2) in gastric cancer development and progression has been extensively studied. The expression of COX-2 is elevated in gastric cancer tissues, therefore, inhibition of COX-2 expression may prevent or reverse gastric carcinogenesis. This review focuses on the crucial role of COX-2 in gastric cancer development and progression. In addition, its mechanisms of action are illustrated.

COX

COX, also known as prostaglandin synthase, is the rate-limiting enzyme responsible for the conversion of arachidonic acid (AA) into the various prostaglandins (PGs), a family of lipid mediators that have widespread and diverse biological functions[5]. This enzyme possesses both peroxidase activity in catalyzing prostaglandin G_2 (PGG_2) to prostaglandin H_2 (PGH_2) and COX catalytic activity in the conversion of PGG_2 from AA[6]. Members of the PGs family including PGD_2, PGE_2, PGF_2, PGG_2, and PGH_2 are widely distributed in organic bodies and play different roles in metabolism[7-11]. It is reported that PGE_2 was overexpressed in tumor tissues and was involved in carcinogenesis[12-14].

COX, with a relative molecular mass of 71000, is a type of glycoprotein which is located on the surface of the nuclear membrane and microsomal membrane. Two COX isoforms have been found: COX-1 and COX-2. Although COX-1 and COX-2 share a high level of homology (65%), the activity and expression of these enzymes are different, and they can function independently within the same cell type[15].

The *COX-1* gene, comprised of 11 exons and 10 introns, is a type of housekeeping gene, which is located at chromosome 9 $q^{32-33.3}$. The full length of the *COX-1* gene is about 22.5 kb, and no hogness box and promoter elements are found. In most tissues, COX-1 is composed of 599-600 amino acid residues and expressed constitutively and continuously[16]. The basic functions of COX-1 are not only promoting the synthesis of PGs, but also maintaining the homeostasis of an organism such as regulating the clotting mechanism, stabilizing renal blood flow and protecting gastric mucosa[17-19]. COX-1 is expressed negatively or weakly in tumor tissues and is not involved in carcinogenesis[20].

The *COX-2* gene, located at chromosome $1q^{25.2-25.3}$, is composed of 10 exons and 9 introns. With hogness box, CAAT/enhancer binding protein (C/EBP) and cAMP response elements in the 5’-terminal nucleotide sequence, the gene is approximately 8.3 kb in size [21]. There are also some binding sites in the gene sequence such as the activator protein-2 (AP-2) binding site and the nuclear factor-kappa B (NF-κB) binding site[22]. COX-2 is composed of 604 amino acid residues and is expressed negatively in normal tissues and organs under physiological conditions, except the constitutive expression in kidney and brain. It is inducible in response to certain stimuli such as growth factors and cytokines. COX-2 is involved in many pathological processes such as inflammation and carcinogenesis[23,24]. It was reported that more than 15% of malignant tumors are correlated with infection[25]. Various inflammation networks have been confirmed to play crucial roles in the microenvironment of carcinogenesis[26], and the most important network is the COX-2/PGE_2 pathway[27]. In addition, it has been well established that COX-2 is up-regulated in a variety of cancers and promotes their growth[28-30].

EXPRESSION OF COX-2 IN GASTRIC CANCER

The first report on the expression of COX-2 in gastric cancer was from Ristimaki *et al*[23]. Their study showed that human gastric adenocarcinoma tissues contained significantly higher levels of COX-2 mRNA when compared with paired gastric mucosal specimens devoid of cancer cells. Immunohistochemical staining detected COX-2 protein expression in the cytoplasm of gastric carcinoma cells, but not in the surrounding stroma. Uefuji confirmed the overexpression of COX-2 protein in human gastric adenocarcinomas by immunoblotting, and reported that overexpression of COX-2 protein was independent of the histologic type of gastric cancer[31]. A study further confirmed the significant difference in COX-2 protein expression between normal tissues and gastric cancer tissues[32]. Researchers found that the overexpression of COX-2 protein was not related to the clinicopathological characteristics of gastric cancer patients[33], but related to tumor node metastasis clinical stage, depth of invasion and metastasis[33,34]. A series of studies showed that COX-2 protein expression was associated with intestinal histological subtype, proximal location, tumor size and advanced clinical stage and lymph node involvement[35-39]. Importantly, the expression of COX-2 protein and mRNA was already detected in noninvasive gastric dysplasia[40,41]. Thus, it seems likely that COX-2 plays a role in early gastric carcinogenesis.

There are controversial results in the association between COX-2 and survival rate. Although COX-2 played a crucial role in gastric carcinogenesis and was relevant to the degree of tumor differentiation, the expression of COX-2 protein was not correlated with survival rate. In addition, it made little sense in predicting gastric cancer prognosis[42]. In contrast, other research results suggested that COX-2 was an independent prognostic factor for gastric cancer as the 5-year survival rate of COX-2 protein positively expressed patients was lower than that of negatively expressed patients[43]. In addition, early-stage gastric cancer patients with high expression of COX-2 protein were at a higher risk for cancer-related death than those with a low level of COX-2 expression[35]. Another study[44] assessed the correlation between tumor progression and epithelial mesenchymal transition using multivariate analysis, and showed that COX-2 protein overexpression was an independent prognostic factor for poor survival, due to angiogenesis, cancer invasion and metastasis. Recently, scientists also found that COX-2 protein and *p53* expression were independent prognostic factors for poor survival, in addition to late-stage disease and non-curative surgery[45]. Most of the findings illustrated above share the similar points, while the controversial points need to be further investigated to reach a consensus.

MECHANISM OF ELEVATED COX-2 EXPRESSION IN GASTRIC CANCER

Helicobacter pylori infection

Some studies suggested that *Helicobacter pylori* (*H. pylori*) infection was significantly related to COX-2 expression[46-48]. *H. pylori* infection can lead to a local inflammatory response, phenotypic change of epithelial cells, promotion of cell proliferation and inhibition of cell apoptosis, and ultimately an increased risk of gastric cancer[49].

H. pylori is classified as a class I carcinogen by the International Agency for Research on Cancer. Over-expression of COX-2 was detected in *H. pylori* positive gastritis compared with *H. pylori* negative gastritis[50]. COX-2 over-expression was found in 50%-80% of gastric cancer patients. Another study showed that 24 h after *H. pylori* infection of epithelial cells in mice, the expression of COX-2 and PGE_2 were significantly elevated[51]. An *in vitro* study also obtained similar results[48]. After the coculture of MKN 28 cell lines with *H. pylori* for 24 h, the COX-2 mRNA transcription level increased five-fold and the expression of PGE_2 increased three-fold, suggesting that synthesis of COX-2 and PGE_2 was one of the factors for *H. pylori* associated gastric cancer[52].

The mechanism of COX-2 over-expression caused by *H. pylori* infection is not entirely clear. In an *in vitro* study, *H. pylori* induced the expression of COX-2 and inducible nitric oxide synthase by activating AP-1 of AGS cells[53]. Cytokines from the *H. pylori* associated inflammatory response also promoted the upregulation of COX-2[54]. In an *in vivo* study, *H. pylori* infection influenced the expression of 385 genes, and 160 of these genes were related to COX-2, including the inflammation genes (*Icam1*), the apoptosis genes (*Clu*), the proliferation genes (*Gdf3*, *Igf2*), the gastric physiology genes (*Galr-1*) and the epithelial barrier function genes (*Tjp1*, *Aqp5*). After treatment with NS398, a COX-2 inhibitor, the expression of 140 genes changed, which indicated that COX-2 was correlated with the occurrence of gastritis[55]. Another study indicated that *H. pylori* could lead to the phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) and its downstream transcription factor ATF-2, and the expression of COX-2 could be inhibited by a p38 MAPK inhibitor. These results indicated that the p38/ATF-2 signal transduction pathway induced by *H. pylori* was the crucial mechanism involved in COX-2 expression[56]. Infection with *H. pylori* stimulated the secretion of gastrin which promoted the expression of COX-2 and extended the half-life of COX-2 mRNA[57]. In addition, the inhibitor of gastrin-releasing peptide decreased the expression of COX-2, which indicated that gastrin may be involved in COX-2 expression induced by *H. pylori*[58].

Suppressor gene mutation

An imbalance of oncogenes and suppressor genes is responsible for carcinogenesis. The mutation of a suppressor gene can lead to the occurrence of cancer. A study revealed that COX-2 expression in patients with *P53* mutation was higher than in those without *P53* mutation. This indicated that *P53* mutation might be related to COX-2 over-expression[59]. The protein of wild-type *P53* could inhibit the formation of a complex composed of TATA box binding proteins and promoters located upstream of the gene sequences, and eventually inhibited the expression of COX-2. In contrast, the product of mutant type *P53* could elevate COX-2 expression by the Ras/Raf/MAPK signal pathway. Moreover, COX-2 could reversibly induce mutation of *P53*, and both were co-expressed in gastric cancer tissues[60].

P16 is a tumor suppressor gene located at chromosome $9p^{21}$. It can inhibit the function of cyclinD1/CDK4 and CDK6 complex, and cause *p53*-independent G1 arrest through the phosphorylation of pRb[61,62]. This gene is usually inactivated in human gastric cancers for different reasons. A study indicated that *p16* was found to harbor promoter methylation associated with the loss of protein expression in cancer cells, suggesting that *p16* inactivation due to promoter methylation may be important for gastric tumorigenesis[63]. Other mechanisms such as mutation and homozygous deletion, are also responsible for the inactivation of *p16*, which lead to the development and progression of tumors[64]. Researchers explored the expression of COX-2 protein and *p16* protein in gastric cancer mucosa, and found that COX-2 protein expression was negatively related to *p16* protein expression. There may be a relationship between the expression of COX-2 and *p16*. However, the mechanism involved needs to be clarified in further research[65,66].

NF-κB

NF-κB is a protein which has the ability to combine with the nucleotide sequence in the promoter region and enhancer region of some genes, and consequently activate or enhance the transcription of these genes. NF-κB is usually distributed in the cytoplasm in the inactive form under physiological conditions. It is then activated and enters the nucleus in response to outside stimuli. The NF-κB signal pathway is involved in many processes such as the inflammatory response, cell proliferation, apoptosis and carcinogenesis. Some inflammatory cytokines, such as tumor necrosis factor (TNF)-α, can activate NF-κB, and this activated transcription factor can induce over-expression of inflammatory factors including COX-2 and TNF-α itself, forming the inflammatory network in the tumor microenvironment[67]. The COX-2 promoter region contains several elements, with the presence of two NF-κB consensus sites[68]. COX-2 expression decreased significantly when NF-κB was blocked by chondroitin sulfate[69,70]. Expression of COX-2 and NF-κB increased simultaneously during the process from chronic atrophic gastritis, dysplasia to gastric cancer. The co-expression of COX-2 and NF-κB played an important role in the angiogenesis of stomach tissues. In addition, NF-κB upregulated the expression of vascular endothelial growth factor (VEGF), which is an important promoter of angiogenesis[71].

MECHANISM OF COX-2 IN GASTRIC CARCINOGENESIS

Cell proliferation and apoptosis

Accumulating evidence indicates that inflammation plays an important role in the development of cancers[72,73]. TNF-α, which is a mediator of PGE_2, plays a crucial role in mediating the inflammatory process through activation of NF-κB. It has been found that stromal NF-κB can enhance proliferation of epithelial cells by inducing cytokines, chemokines, and growth factors, such as IL-6, IL-1β, macrophage inflammatory protein-2 and TNF-α, while epithelial NF-κB can suppress apoptosis by inducing anti-apoptotic proteins, such as GADD45β, A1/Bfl1, and cIAP1[74,75]. As a product of AA catalyzed by COX-2, PGE_2 is involved in gene mutation and cancer cell proliferation[76]. Protein encoded by *COX-2* genes is a type of oncogenic protein, which could promote the high expression of PGE_2. PGE_2 can stimulate the growth of blood vessels, inhibit local immune function and regulate a variety of signal transduction pathways which ultimately influence the proliferation of cells and the growth of tumors[77]. It has also been confirmed that over-expression of COX-2 promoted cell proliferation by weakening the anti-proliferative effect of transforming growth factor-β (TGF-β)[78].

An *in vitro* study showed that cell proliferation was suppressed when gastric cells were treated with COX-2 small interfering RNA (siRNA)[79]. In MKN-45 cells, inhibition of COX-2 with NS-398 led to reduced proliferation and induction of apoptosis, connected with downregulation of *Bcl-2* (an anti-apoptotic gene) and upregulation of *Bax* (an apoptotic gene)[80]. COX-2 was a regulatory factor in the *Bcl-2* upstream sequences, which upregulated the expression of *Mcl-1*, a member of the *Bcl-2* family, through the phosphatidyl inositol 3-kinase (PI3K) signal pathway, and eventually inhibited the apoptosis of cancer cells[81]. Some studies confirmed that COX-2 could inhibit the apoptosis of cancer cells by inducing the mutation of *P53*[82]. Other researchers indicated that COX-2 weakened the apoptotic signal mediated by *Fas* protein. After adding the COX-2 inhibitor, they detected the elevated expression of caspase, a key enzyme of death receptor signaling and apoptotic signaling pathways[83].

Angiogenesis and lymphatic metastasis

The growth and metastasis of tumors depend on the formation of new blood vessels. PGE_2 and PGF_2 can promote vessel formation directly or indirectly[84]. Research has found that COX-2 over-expression was associated with increased PGE_2 biosynthesis and angiogenesis in gastric cancer[85]. Furthermore, COX-2 can induce cells to produce VEGF and TGF-β, which could promote endotheliocyte migration and tubular morphogenesis. In addition, VEGF was an independent prognostic factor for gastric cancer prognosis[86]. After being transfected with COX-2 siRNA, the expression of VEGF was down-regulated and the growth of gastric cancer cells was significantly inhibited[22]. Other studies indicated that COX-2 upregulated the expression of *Bcl-2* and *Akt*, which can inhibit the apoptosis of endothelial cells and promote vessel formation[87,88].

VEGF-C has been identified as a new member of the VEGF family and is considered a specific lymphangiogenic factor. It can promote the formation and dilation of lymphatic vessels, enhance the permeability of lymphatic vessels and facilitate lymphatic metastasis. A recent study confirmed a positive correlation between the expression of COX-2 and VEGF-C in gastric cancer patients, and both were related to gastric cancer prognosis[89].

Invasion and metastasis

Invasion is the premise of cancer metastasis which involves a variety of cytokines. Adhesion molecule is a type of cell surface glycoprotein that mediates cell adhesion. E-cadherin adhesion which inhibits the separation of cancer cells from tissue can prevent cancer cell invasion. COX-2 can lower the activity of E-cadherin, thus the invasiveness of cancer cells is enhanced for further metastasis. The activity of E-cadherin was enhanced after inhibition of COX-2[90]. It has been confirmed that the over-expression of matrix metalloproteinase (MMP) accelerated the decomposition of collagen in local tissues, which was beneficial to the spread of cancer cells. A correlation between COX-2 and MMP upregulation was also found[91]. COX-2 inhibitors reduce the expression of MMP[92]. CD44, which acts as the membrane receptor of hyaluronic acid and is expressed in cancer stem-like cells, played an important role in cancer metastasis. A large number of CD44(+) gastric glands was found in human adenocarcinomas and adjacent metaplasias, but not in normal gastric epithelium. In addition, CD44(+) tumor cell expansion is triggered by the cooperative actions of PGE_2 and Wnt in gastric tumorigenesis[93]. Studies also confirmed that PGE_2 could upregulate the expression of CD44[94], while COX-2 inhibitor could inhibit CD44 expression[95]. A study demonstrated that CD44v, a variant form of CD44, could protect tumor cells from oxidative stress in a mouse gastric cancer model, thus it plays an important role in tumor development[96]. Other possible mechanisms include the upregulation of urokinase-type plasminogen activator (uPA) in promoting the metastasis of cancer cells[97] and more potential pathways need to be further clarified.

Immunosuppression

It has been found that COX-2 was involved in the immunosuppression in gastric cancer, where effector T cells were suppressed by regulatory T cells. In Treg cells, expression of COX-2 was correlated with that of forkhead box p3. By using a COX-2 inhibitor, the immunosuppression of effector T cells was reversed[98]. The possible mechanisms involved may be as follows: PGE_2 disabled the function of dendritic cells in the tumor microenvironment and the cells could not present the tumor antigen effectively, and eventually the T cells did not recognize or kill the cancer cells[99]. In addition, PGE_2 may also reduce the immunosurveillance of the immune system on mutant cells by inhibiting the expression of human leucocyte antigen Ⅰ and Ⅱ, and by reducing the production of lymphokine. The immunosurveillance effect could be enhanced by using a COX-2 inhibitor and stimulating the activity of natural killer cells[100].

CONCLUSION

The COX-2/PGE_2 pathway involved in the inflammatory response plays a critical role in the microenvironment of gastric tumorigenesis. Expression of COX-2 is elevated in gastric cancer and its over-expression is associated with *H. pylori* infection, mutation of suppressor genes and NF-κB. Over-expressed COX-2 participates in gastric carcinogenesis by promoting cell proliferation, inhibiting cell apoptosis, inducing vessel formation, and enhancing metastasis and immunosuppression. Although progress has been made in exploring the mechanism of gastric cancer development, some issues remain to be explored in further studies. As research continues, interventions in gastric cancer using COX-2 as a target might eventually become a specific treatment of choice.

REFERENCES

1 **Bornschein J**, Rokkas T, Selgrad M, Malfertheiner P. Gastric cancer: clinical aspects, epidemiology and molecular background. *Helicobacter* 2011; **16** Suppl 1: 45-52 [PMID: 21896085 DOI: 10.1111/j.1523-5378.2011.00880.x]

2 **Jing JJ**, Liu HY, Hao JK, Wang LN, Wang YP, Sun LH, Yuan Y. Gastric cancer incidence and mortality in Zhuanghe, China, between 2005 and 2010. *World J Gastroenterol* 2012; **18**: 1262-1269 [PMID: 22468091 DOI: 10.3748/wjg.v18.i11.1262]

3 **Krejs GJ**. Gastric cancer: epidemiology and risk factors. *Dig Dis* 2010; **28**: 600-603 [PMID: 21088409 DOI: 10.1159/000320277]

4 **Lin Y**, Ueda J, Kikuchi S, Totsuka Y, Wei WQ, Qiao YL, Inoue M. Comparative epidemiology of gastric cancer between Japan and China. *World J Gastroenterol* 2011; **17**: 4421-4428 [PMID: 22110269 DOI: 10.3748/wjg.v17.i39.4421]

5 **Smyth EM**, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. *J Lipid Res* 2009; **50** Suppl: S423-S428 [PMID: 19095631 DOI: 10.1194/jlr.R800094-JLR200]

6 **Yu Q**, Purwaha P, Ni K, Sun C, Mallik S, Qian SY. Characterization of novel radicals from COX-catalyzed arachidonic acid peroxidation. *Free Radic Biol Med* 2009; **47**: 568-576 [PMID: 19482075 DOI: 10.1016/j.freeradbiomed.2009]

7 **Ruan YC**, Zhou W, Chan HC. Regulation of smooth muscle contraction by the epithelium: role of prostaglandins. *Physiology* (Bethesda) 2011; **26**: 156-170 [PMID: 21670162 DOI: 10.1152/physiol.00036.2010]

8 **Hein AM**, O'Banion MK. Neuroinflammation and memory: the role of prostaglandins. *Mol Neurobiol* 2009; **40**: 15-32 [PMID: 19365736 DOI: 10.1007/s12035-009-8066-z]

9 **Stratton R**, Shiwen X. Role of prostaglandins in fibroblast activation and fibrosis. *J Cell Commun Signal* 2010; **4**: 75-77 [PMID: 20531982 DOI: 10.1007/s12079-010-0089-8]

10 **Gibb W**. The role of prostaglandins in human parturition. *Ann Med* 1998; **30**: 235-241 [PMID: 9677008 DOI: 10.3109/07853899809005850]

11 **Wang D**, Mann JR, DuBois RN. The role of prostaglandins and other eicosanoids in the gastrointestinal tract. *Gastroenterology* 2005; **128**: 1445-1461 [PMID: 15887126 DOI: 10.1053/j.gastro.2004.09.080]

12 **Brown JR**, DuBois RN. COX-2: a molecular target for colorectal cancer prevention. *J Clin Oncol* 2005; **23**: 2840-2855 [PMID: 15837998 DOI: 10.1200/jco.2005.09.051]

13 **Hogendorf P**, Durczyński A, Kumor A, Strzelczyk J. Prostaglandin E2 (PGE2) in portal blood in patients with pancreatic tumor--a single institution series. *J Invest Surg* 2012; **25**: 8-13 [PMID: 22272632 DOI: 10.3109/08941939.2011.592569]

14 **Li J**, Feng G, Liu J, Rong R, Luo F, Guo L, Zhu T, Wang G, Chu Y. Renal cell carcinoma may evade the immune system by converting CD4+Foxp3- T cells into CD4+CD25+Foxp3+ regulatory T cells: Role of tumor COX-2-derived PGE2. *Mol Med Rep* 2010; **3**: 959-963 [PMID: 21472340 DOI: 10.3892/mmr.2010.374]

15 **Davidge ST**. Prostaglandin H synthase and vascular function. *Circ Res* 2001; **89**: 650-660 [PMID: 11597987 DOI: 10.1161/hh2001.098351]

16 **Bamba H**, Ota S, Arai S, Ban S, Shimizu M, Imai Y, Kawamoto C, Yoshida Y, Fujiwara K. Expression of cyclooxygenase-2 in human hyperplastic gastric polyps. *J Exp Clin Cancer Res* 2003; **22**: 425-430 [PMID: 14582702]

17 **Peskar BM**, Sawka N, Ehrlich K, Peskar BA. Role of cyclooxygenase-1 and -2, phospholipase C, and protein kinase C in prostaglandin-mediated gastroprotection. *J Pharmacol Exp Ther* 2003; **305**: 1233-1238 [PMID: 12626649 DOI: 10.1124/jpet.103.049650]

18 **Tokuyama H**, Hayashi K, Matsuda H, Kubota E, Honda M, Okubo K, Ozawa Y, Saruta T. Distinct role of intrarenal cyclooxygenase-1/2 in chronic unilateral renal ischemia. *Nephron* 2002; **92**: 183-191 [PMID: 12187101 DOI: 10.1159/000064479]

19 **Lipsky PE**. Role of cyclooxygenase-1 and -2 in health and disease. *Am J Orthop* (Belle Mead NJ) 1999; **28**: 8-12 [PMID: 10193997]

20 **Saukkonen K**, Rintahaka J, Sivula A, Buskens CJ, Van Rees BP, Rio MC, Haglund C, Van Lanschot JJ, Offerhaus GJ, Ristimaki A. Cyclooxygenase-2 and gastric carcinogenesis. *APMIS* 2003; **111**: 915-925 [PMID: 14616542 DOI: 10.1034/j.1600-0463.2003.1111001.x]

21 **Sonoda R**, Naomoto Y, Shirakawa Y, Fujiwara Y, Yamatsuji T, Noma K, Tanabe S, Takaoka M, Gunduz M, Tsujigiwa H, Nagatsuka H, Ohara N, Yoshino T, Takubo K, Vieth M, Tanaka N. Preferential up-regulation of heparanase and cyclooxygenase-2 in carcinogenesis of Barrett's oesophagus and intestinal-type gastric carcinoma. *Histopathology* 2010; **57**: 90-100 [PMID: 20653782 DOI: 10.1111/j.1365-2559.2010.03594.x]

22 **Yao L**, Liu F, Hong L, Sun L, Liang S, Wu K, Fan D. The function and mechanism of COX-2 in angiogenesis of gastric cancer cells. *J Exp Clin Cancer Res* 2011; **30**: 13 [PMID: 21266034 DOI: 10.1186/1756-9966-30-13]

23 **Ristimäki A**, Honkanen N, Jänkälä H, Sipponen P, Härkönen M. Expression of cyclooxygenase-2 in human gastric carcinoma. *Cancer Res* 1997; **57**: 1276-1280 [PMID: 9102213]

24 **Kargman S**, Charleson S, Cartwright M, Frank J, Riendeau D, Mancini J, Evans J, O'Neill G. Characterization of Prostaglandin G/H Synthase 1 and 2 in rat, dog, monkey,

and human gastrointestinal tracts. *Gastroenterology* 1996; **111**: 445-454 [PMID: 8690211 DOI: 10.1053/gast.1996.v111.pm8690211]

25 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044 [PMID: 16404738 DOI: 10.1002/ijc.21731]

26 **Grivennikov SI**, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883-899 [PMID: 20303878 DOI: 10.1016/j.cell.2010.01.025]

27 **Wang D**, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* 2010; **29**: 781-788 [PMID: 19946329 DOI: 10.1038/onc.2009.421]

28 **de Heer P**, Gosens MJ, de Bruin EC, Dekker-Ensink NG, Putter H, Marijnen CA, van den Brule AJ, van Krieken JH, Rutten HJ, Kuppen PJ, van de Velde CJ. Cyclooxygenase 2 expression in rectal cancer is of prognostic significance in patients receiving preoperative radiotherapy. *Clin Cancer Res* 2007; **13**: 2955-2960 [PMID: 17504996 DOI: 10.1158/1078-0]

29 **Higashi Y**, Kanekura T, Kanzaki T. Enhanced expression of cyclooxygenase (COX)-2 in human skin epidermal cancer cells: evidence for growth suppression by inhibiting COX-2 expression. *Int J Cancer* 2000; **86**: 667-671 [PMID: 10797288 DOI: 10.1002/(sici)1097-0215(20000601)86:5]

30 **Farooqui M**, Li Y, Rogers T, Poonawala T, Griffin RJ, Song CW, Gupta K. COX-2 inhibitor celecoxib prevents chronic morphine-induced promotion of angiogenesis, tumour growth, metastasis and mortality, without compromising analgesia. *Br J Cancer* 2007; **97**: 1523-1531 [PMID: 17971769 DOI: 10.1038/sj.bjc.6604057]

31 **Uefuji K**, Ichikura T, Mochizuki H, Shinomiya N. Expression of cyclooxygenase-2 protein in gastric adenocarcinoma. *J Surg Oncol* 1998; **69**: 168-172 [PMID: 9846504 DOI: 10.1002/(SICI)1096-9098(199811)]

32 **Lim HY**, Joo HJ, Choi JH, Yi JW, Yang MS, Cho DY, Kim HS, Nam DK, Lee KB, Kim HC. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. *Clin Cancer Res* 2000; **6**: 519-525 [PMID: 10690533]

33 **Mao XY**, Wang XG, Lv XJ, Xu L, Han CB. COX-2 expression in gastric cancer and its relationship with angiogenesis using tissue microarray. *World J Gastroenterol* 2007; **13**: 3466-3471 [PMID: 17659693]

34 **Murata H**, Kawano S, Tsuji S, Tsuji M, Sawaoka H, Kimura Y, Shiozaki H, Hori M. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. *Am J Gastroenterol* 1999; **94**: 451-455 [PMID: 10022645 DOI: 10.1111/j.1572-0241.1999.876_e.x]

35 **Mrena J**, Wiksten JP, Thiel A, Kokkola A, Pohjola L, Lundin J, Nordling S, Ristimäki A, Haglund C. Cyclooxygenase-2 is an independent prognostic factor in gastric cancer and its expression is regulated by the messenger RNA stability factor HuR. *Clin Cancer Res* 2005; **11**: 7362-7368 [PMID: 16243808 DOI: 10.1158/1078-0432.ccr-05-0764]

36 **Yu HG**, Li JY, Yang YN, Luo HS, Yu JP, Meier JJ, Schrader H, Bastian A, Schmidt WE, Schmitz F. Increased abundance of cyclooxygenase-2 correlates with vascular endothelial growth factor-A abundance and tumor angiogenesis in gastric cancer. *Cancer Lett* 2003; **195**: 43-51 [PMID: 12767510 DOI: 10.1016/S0304-3835(03)00153-8]

37 **Da MX**, Wu XT, Wang J, Guo TK, Zhao ZG, Luo T, Zhang MM, Qian K. Expression of cyclooxygenase-2 and vascular endothelial growth factor-C correlates with lymphangiogenesis and lymphatic invasion in human gastric cancer. *Arch Med Res* 2008; **39**: 92-99 [PMID: 18068001 DOI: 10.1016/j.arcmed.2007.06.021]

38 **Li M**, Liu W, Zhu YF, Chen YL, Zhang BZ, Wang R. Correlation of COX-2 and K-ras expression to clinical outcome in gastric cancer. *Acta Oncol* 2006; **45**: 1115-1119 [PMID: 17118848 DOI: 10.1088/02841860601043066]

39 **Al-Moundhri MS**, Al-Hadabi I, Al-Mawaly K, Kumar S, Al-Lawati FA, Bhatnager G, Kuruvila S, Al-Hamdani A, El-Sayed SM, Al-Bahrani B. Prognostic significance of cyclooxygenase-2, epidermal growth factor receptor 1, and microvascular density in gastric cancer. *Med Oncol* 2012; **29**: 1739-1747 [PMID: 22048943 DOI: 10.1007/s12032-011-0098-3]

40 **Oba M**, Miwa K, Fujimura T, Harada S, Sasaki S, Hattori T. Chemoprevention of glandular stomach carcinogenesis through duodenogastric reflux in rats by a COX-2 inhibitor. *Int J Cancer* 2008; **123**: 1491-1498 [PMID: 18646190 DOI: 10.1002/ijc.23742]

41 **van Rees BP**, Saukkonen K, Ristimäki A, Polkowski W, Tytgat GN, Drillenburg P, Offerhaus GJ. Cyclooxygenase-2 expression during carcinogenesis in the human stomach. *J Pathol* 2002; **196**: 171-179 [PMID: 11793368 DOI: 10.1002/path.1033]

42 **Joo YE**, Oh WT, Rew JS, Park CS, Choi SK, Kim SJ. Cyclooxygenase-2 expression is associated with well-differentiated and intestinal-type pathways in gastric carcinogenesis. *Digestion* 2002; **66**: 222-229 [PMID: 12592098 DOI: 10.1159/000068366]

43 **Han SL**, Tang HJ, Hua YW, Ji SQ, Lin DX. Expression of COX-2 in stomach cancers and its relation to their biological features. *Dig Surg* 2003; **20**: 107-114 [PMID: 12686777 DOI: 10.1159/000069384]

44 **Fanelli MF**, Chinen LT, Begnami MD, Costa WL, Fregnami JH, Soares FA, Montagnini AL. The influence of transforming growth factor-α, cyclooxygenase-2, matrix metalloproteinase (MMP)-7, MMP-9 and CXCR4 proteins involved in epithelial-mesenchymal transition on overall survival of patients with gastric cancer. *Histopathology* 2012; **61**: 153-161 [PMID: 22582975 DOI: 10.1111/j.1365-2559.2011.04139.x]

45 **Mrena J**, Wiksten JP, Kokkola A, Nordling S, Ristimäki A, Haglund C. COX-2 is associated with proliferation and apoptosis markers and serves as an independent prognostic factor in gastric cancer. *Tumour Biol* 2010; **31**: 1-7 [PMID: 20237896 DOI: 10.1007/s13277-009-0001-4]

46 **Zhang LJ**, Wang SY, Huo XH, Zhu ZL, Chu JK, Ma JC, Cui DS, Gu P, Zhao ZR, Wang MW, Yu J. Anti-Helicobacter pylori therapy followed by celecoxib on progression of gastric precancerous lesions. *World J Gastroenterol* 2009; **15**: 2731-2738 [PMID: 19522023 DOI: 10.3748/wjg.15.2731]

47 **Pero R**, Peluso S, Angrisano T, Tuccillo C, Sacchetti S, Keller S, Tomaiuolo R, Bruni CB, Lembo F, Chiariotti L. Chromatin and DNA methylation dynamics of Helicobacter pylori-induced COX-2 activation. *Int J Med Microbiol* 2011; **301**: 140-149 [PMID: 20934379 DOI: 10.1016/j.ijmm.2010.06.009]

48 **Targosz A**, Brzozowski T, Pierzchalski P, Szczyrk U, Ptak-Belowska A, Konturek SJ, Pawlik W. Helicobacter pylori promotes apoptosis, activates cyclooxygenase (COX)-2 and inhibits heat shock protein HSP70 in gastric cancer epithelial cells. *Inflamm Res* 2012; **61**: 955-966 [PMID: 22610150 DOI: 10.1007/s00011-012-0487-x]

49 **Asaka M**. Helicobacter pylori infection and gastric cancer. *Intern Med* 2002; **41**: 1-6 [PMID: 11838583 DOI: 10.2169/internalmedicine.41.1]

50 **Fu S**, Ramanujam KS, Wong A, Fantry GT, Drachenberg CB, James SP, Meltzer SJ, Wilson KT. Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in Helicobacter pylori gastritis. *Gastroenterology* 1999; **116**: 1319-1329 [PMID: 10348815 DOI: 10.1016/S0016-5085(99)70496-8]

51 **Xiao F**, Furuta T, Takashima M, Shirai N, Hanai H. Involvement of cyclooxygenase-2 in hyperplastic gastritis induced by Helicobacter pylori infection in C57BL/6 mice. *Aliment Pharmacol Ther* 2001; **15**: 875-886 [PMID: 11380326 DOI: 10.1046/j.1365-2036.2001.00965.x]

52 **Romano M**, Ricci V, Memoli A, Tuccillo C, Di Popolo A, Sommi P, Acquaviva AM, Del Vecchio Blanco C, Bruni CB, Zarrilli R. Helicobacter pylori up-regulates cyclooxygenase-2 mRNA expression and prostaglandin E2 synthesis in

MKN 28 gastric mucosal cells in vitro. *J Biol Chem* 1998; **273**: 28560-28563 [PMID: 9786845 DOI: 10.1074/jbc.273.44.28560]

53 **Cho SO**, Lim JW, Kim KH, Kim H. Involvement of Ras and AP-1 in Helicobacter pylori-induced expression of COX-2 and iNOS in gastric epithelial AGS cells. *Dig Dis Sci* 2010; **55**: 988-996 [PMID: 19495976 DOI: 10.1007/s10620-009-0828-y]

54 **Tokudome S**, Ghadimi R, Suzuki S, Hosono A, Tanaka T, Arakawa K, Zhao Y, Hattori N, Agawa H, Marumoto M, Ando R, Moore MA. Helicobacter pylori infection appears the prime risk factor for stomach cancer. *Int J Cancer* 2006; **119**: 2991; author reply 2992 [PMID: 17016826 DOI: 10.1002/ijc.22223]

55 **Walduck AK**, Weber M, Wunder C, Juettner S, Stolte M, Vieth M, Wiedenmann B, Meyer TF, Naumann M, Hoecker M. Identification of novel cyclooxygenase-2-dependent genes in Helicobacter pylori infection in vivo. *Mol Cancer* 2009; **8**: 22 [PMID: 19317916 DOI: 10.1186/1476-4598-8-22]

56 **Li Q**, Liu N, Shen B, Zhou L, Wang Y, Wang Y, Sun J, Fan Z, Liu RH. Helicobacter pylori enhances cyclooxygenase 2 expression via p38MAPK/ATF-2 signaling pathway in MKN45 cells. *Cancer Lett* 2009; **278**: 97-103 [PMID: 19201083 DOI: 10.1016/j.canlet.2008.12.032]

57 **Subramaniam D**, Ramalingam S, May R, Dieckgraefe BK, Berg DE, Pothoulakis C, Houchen CW, Wang TC, Anant S. Gastrin-mediated interleukin-8 and cyclooxygenase-2 gene expression: differential transcriptional and posttranscriptional mechanisms. *Gastroenterology* 2008; **134**: 1070-1082 [PMID: 18395088 DOI: 10.1053/j.gastro.2008.01.040]

58 **Hohla F**, Schally AV, Kanashiro CA, Buchholz S, Baker B, Kannadka C, Moder A, Aigner E, Datz C, Halmos G. Growth inhibition of non-small-cell lung carcinoma by BN/GRP antagonist is linked with suppression of K-Ras, COX-2, and pAkt. *Proc Natl Acad Sci USA* 2007; **104**: 18671-18676 [PMID: 18003891 DOI: 10.1073/pnas.0709455104]

59 **Saukkonen K**, Nieminen O, van Rees B, Vilkki S, Härkönen M, Juhola M, Mecklin JP, Sipponen P, Ristimäki A. Expression of cyclooxygenase-2 in dysplasia of the stomach and in intestinal-type gastric adenocarcinoma. *Clin Cancer Res* 2001; **7**: 1923-1931 [PMID: 11448905]

60 **Hermanova M**, Trna J, Nenutil R, Dite P, Kala Z. Expression of COX-2 is associated with accumulation of p53 in pancreatic cancer: analysis of COX-2 and p53 expression in premalignant and malignant ductal pancreatic lesions. *Eur J Gastroenterol Hepatol* 2008; **20**: 732-739 [PMID: 18617777 DOI: 10.1097/MEG.0b013e3282f945fb]

61 **Lukas J**, Parry D, Aagaard L, Mann DJ, Bartkova J, Strauss M, Peters G, Bartek J. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. *Nature* 1995; **375**: 503-506 [PMID: 7777060 DOI: 10.1038/375503a0]

62 **Serrano M**, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 1993; **366**: 704-707 [PMID: 8259215 DOI: 10.1038/366704a0]

63 **Goto T**, Mizukami H, Shirahata A, Yokomizo K, Kitamura YH, Sakuraba K, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K. Methylation of the p16 gene is frequently detected in lymphatic-invasive gastric cancer. *Anticancer Res* 2010; **30**: 2701-2703 [PMID: 20683001]

64 **Kobayashi N**, Toyooka S, Yanai H, Soh J, Fujimoto N, Yamamoto H, Ichihara S, Kimura K, Ichimura K, Sano Y, Kishimoto T, Date H. Frequent p16 inactivation by homozygous deletion or methylation is associated with a poor prognosis in Japanese patients with pleural mesothelioma. *Lung Cancer* 2008; **62**: 120-125 [PMID: 18395929 DOI: 10.1016/j.lungcan.2008.02.013]

65 **Li Q**, Gao SL, Cui LJ, Li MQ. Expression of COX-2 and P16 protein and their correlations in various types of gastric polyps and gastric cancer cell. *Shijie Huaren Xiaohua Zazhi* 2005; **13**: 2506-2509 [DOI: 10.3969/j.issn.1009-3079.2005.20.025]

66 **Zhai JS**, Li N, Zhang L, Wang YM, Zhu CH, Wu K. Expression of P16,C-MYC and COX-2 in Gastric Carcinoma and Relationship with the Prognosis of Patients. *Zhejiang Zhongyiyao Daxue Xuebao* 2009; **33**: 321-322 [DOI: 10.3969/j.issn.1005-5509.2009.03.012]

67 **Oshima H**, Oshima M. The inflammatory network in the gastrointestinal tumor microenvironment: lessons from mouse models. *J Gastroenterol* 2012; **47**: 97-106 [PMID: 22218775 DOI: 10.1007/s00535-011-0523-6]

68 **Lim JW**, Kim H, Kim KH. NF-kappaB, inducible nitric oxide synthase and apoptosis by Helicobacter pylori infection. *Free Radic Biol Med* 2001; **31**: 355-366 [PMID: 11461773 DOI: 10.1016/S0891-5849(01)00592-5]

69 **Xu CX**, Jin H, Chung YS, Shin JY, Lee KH, Beck GR, Palmos GN, Choi BD, Cho MH. Chondroitin sulfate extracted from ascidian tunic inhibits phorbol ester-induced expression of Inflammatory factors VCAM-1 and COX-2 by blocking NF-kappaB activation in mouse skin. *J Agric Food Chem* 2008; **56**: 9667-9675 [PMID: 18800802 DOI: 10.1021/jf801578]

70 **Li YS**, Wu LP, Li KH, Liu YP, Xiang R, Zhang SB, Zhu LY, Zhang LY. Involvement of nuclear factor κB (NF-κB) in the downregulation of cyclooxygenase-2 (COX-2) by genistein in gastric cancer cells. *J Int Med Res* 2011; **39**: 2141-2150 [PMID: 22289529 DOI: 10.1177/147323001103900610]

71 **Nam SY**, Ko YS, Jung J, Yoon J, Kim YH, Choi YJ, Park JW, Chang MS, Kim WH, Lee BL. A hypoxia-dependent upregulation of hypoxia-inducible factor-1 by nuclear factor-κB promotes gastric tumour growth and angiogenesis. *Br J Cancer* 2011; **104**: 166-174 [PMID: 21119667 DOI: 10.1038/sj.bjc.6606020]

72 **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867 [PMID: 12490959 DOI: 10.1038/nature01322]

73 **Mantovani A**, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454**: 436-444 [PMID: 18650914 DOI: 10.1038/nature07205]

74 **Pikarsky E**, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* 2004; **431**: 461-466 [PMID: 15329734 DOI: 10.1038/nature02924]

75 **Greten FR**, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004; **118**: 285-296 [PMID: 15294155 DOI: 10.1016/j.cell.2004.07.013]

76 **Akhtar M**, Cheng Y, Magno RM, Ashktorab H, Smoot DT, Meltzer SJ, Wilson KT. Promoter methylation regulates Helicobacter pylori-stimulated cyclooxygenase-2 expression in gastric epithelial cells. *Cancer Res* 2001; **61**: 2399-2403 [PMID: 11289104]

77 **Moalic S**, Liagre B, Le Bail JC, Beneytout JL. Dose-dependent modulation of apoptosis and cyclooxygenase-2 expression in human 1547 osteosarcoma cells by NS-398, a selective cyclooxygenase-2 inhibitor. *Int J Oncol* 2001; **18**: 533-540 [PMID: 11179483]

78 **Enders GA**. Cyclooxygenase-2 overexpression abrogates the antiproliferative effects of TGF-beta. *Br J Cancer* 2007; **97**: 1388-1392 [PMID: 17955050 DOI: 10.1038/sj.bjc.6604048]

79 **Fu YG**, Sung JJ, Wu KC, Wu HP, Yu J, Chan M, Chan VY, Chan KK, Fan DM, Leung WK. Inhibition of gastric cancer-associated angiogenesis by antisense COX-2 transfectants. *Cancer Lett* 2005; **224**: 243-252 [PMID: 15914275 DOI: 10.1016/j.canlet.2004.11.038]

80 **Sun WH**, Zhu F, Chen GS, Su H, Luo C, Zhao QS, Zhang Y, Shao Y, Sun J, Zhou SM, Ding GX, Cheng YL. Blockade of cholecystokinin-2 receptor and cyclooxygenase-2 synergistically induces cell apoptosis, and inhibits the proliferation of human gastric cancer cells in vitro. *Cancer Lett* 2008; **263**: 302-311 [PMID: 18258354 DOI: 10.1016/j.canlet.2008.01.012]

81 **Chen XL**, Su BS, Sun RQ, Zhang J, Wang YL. Relationship between expression and distribution of cyclooxygenase-2 and bcl-2 in human gastric adenocarcinoma. *World J Gastroenterol* 2005; **11**: 1228-1231 [PMID: 15754411]

82 **Han JA**, Kim JI, Ongusaha PP, Hwang DH, Ballou LR, Mahale A, Aaronson SA, Lee SW. P53-mediated induction of Cox-2 counteracts p53- or genotoxic stress-induced apoptosis. *EMBO J* 2002; **21**: 5635-5644 [PMID: 12411481 DOI: 10.1093/emboj/cdf591]

83 **Casado M**, Mollá B, Roy R, Fernández-Martínez A, Cucarella C, Mayoral R, Boscá L, Martín-Sanz P. Protection against Fas-induced liver apoptosis in transgenic mice expressing cyclooxygenase 2 in hepatocytes. *Hepatology* 2007; **45**: 631-638 [PMID: 17326157 DOI: 10.1002/hep.21556]

84 **Bamba H**, Ota S, Kato A, Kawamoto C, Fujiwara K. Prostaglandins up-regulate vascular endothelial growth factor production through distinct pathways in differentiated U937 cells. *Biochem Biophys Res Commun* 2000; **273**: 485-491 [PMID: 10873632 DOI: 10.1006/bbrc.2000.2969]

85 **Uefuji K**, Ichikura T, Mochizuki H. Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. *Clin Cancer Res* 2000; **6**: 135-138 [PMID: 10656441]

86 **Vidal O**, Soriano-Izquierdo A, Pera M, Elizalde JI, Palacín A, Castells A, Piqué JM, Volant A, Metges JP. Positive VEGF immunostaining independently predicts poor prognosis in curatively resected gastric cancer patients: results of a study assessing a panel of angiogenic markers. *J Gastrointest Surg* 2008; **12**: 1005-1014 [PMID: 17972143 DOI: 10.1007/s11605-007-0336-3]

87 **Kim N**, Kim CH, Ahn DW, Lee KS, Cho SJ, Park JH, Lee MK, Kim JS, Jung HC, Song IS. Anti-gastric cancer effects of celecoxib, a selective COX-2 inhibitor, through inhibition of Akt signaling. *J Gastroenterol Hepatol* 2009; **24**: 480-487 [PMID: 18823436 DOI: 10.1111/j.1440-1746.2008.05599.x]

88 **Pidgeon GP**, Barr MP, Harmey JH, Foley DA, Bouchier-Hayes DJ. Vascular endothelial growth factor (VEGF) upregulates BCL-2 and inhibits apoptosis in human and murine mammary adenocarcinoma cells. *Br J Cancer* 2001; **85**: 273-278 [PMID: 11461089 DOI: 10.1054/bjoc.2001.1876]

89 **Gou HF**, Chen XC, Zhu J, Jiang M, Yang Y, Cao D, Hou M. Expressions of COX-2 and VEGF-C in gastric cancer: correlations with lymphangiogenesis and prognostic implications. *J Exp Clin Cancer Res* 2011; **30**: 14 [PMID: 21272377 DOI: 10.1186/1756-9966-30-14]

90 **Rao DS**, Gui D, Koski ME, Popoviciu LM, Wang H, Reiter RE, Said JW. An inverse relation between COX-2 and E-cadherin expression correlates with aggressive histologic features in prostate cancer. *Appl Immunohistochem Mol Morphol* 2006; **14**: 375-383 [PMID: 17122632 DOI: 10.1097/01.pai.0000210417.61117.6c]

91 **Dicken BJ**, Graham K, Hamilton SM, Andrews S, Lai R, Listgarten J, Jhangri GS, Saunders LD, Damaraju S, Cass C. Lymphovascular invasion is associated with poor survival in gastric cancer: an application of gene-expression and tissue array techniques. *Ann Surg* 2006; **243**: 64-73 [PMID: 16371738 DOI: 10.1097/01.sla.0000194087.96582.3e]

92 **Zhao Y**, Zhou S, Heng CK. Celecoxib inhibits serum amyloid a-induced matrix metalloproteinase-10 expression in human endothelial cells. *J Vasc Res* 2009; **46**: 64-72 [PMID: 18552508 DOI: 10.1159/000139134]

93 **Ishimoto T**, Oshima H, Oshima M, Kai K, Torii R, Masuko T, Baba H, Saya H, Nagano O. CD44+ slow-cycling tumor cell expansion is triggered by cooperative actions of Wnt and prostaglandin E2 in gastric tumorigenesis. *Cancer Sci* 2010; **101**: 673-678 [PMID: 20028388 DOI: 10.1111/j.1349-7006.2009.01430.x]

94 **Dohadwala M**, Batra RK, Luo J, Lin Y, Krysan K, Pold M, Sharma S, Dubinett SM. Autocrine/paracrine prostaglandin E2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclooxygenase-2-dependent invasion. *J Biol Chem* 2002; **277**: 50828-50833 [PMID: 12393872 DOI: 10.1074/jbc.M210707200]

95 **Jia XQ**, Zhong N, Han LH, Wang JH, Yan M, Meng FL, Zhang SZ. Effect of NS-398 on colon cancer cells. *World J Gastroenterol* 2005; **11**: 353-356 [PMID: 15637743]

96 **Ishimoto T**, Nagano O, Yae T, Tamada M, Motohara T, Oshima H, Oshima M, Ikeda T, Asaba R, Yagi H, Masuko T, Shimizu T, Ishikawa T, Kai K, Takahashi E, Imamura Y, Baba Y, Ohmura M, Suematsu M, Baba H, Saya H. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. *Cancer Cell* 2011; **19**: 387-400 [PMID: 21397861 DOI: 10.1016/j.ccr.2011.01.038]

97 **Shin VY**, Wu WK, Chu KM, Wong HP, Lam EK, Tai EK, Koo MW, Cho CH. Nicotine induces cyclooxygenase-2 and vascular endothelial growth factor receptor-2 in association with tumor-associated invasion and angiogenesis in gastric cancer. *Mol Cancer Res* 2005; **3**: 607-615 [PMID: 16317086 DOI: 10.1158/1541-7786.mcr-05-0106]

98 **Yuan XL**, Chen L, Li MX, Dong P, Xue J, Wang J, Zhang TT, Wang XA, Zhang FM, Ge HL, Shen LS, Xu D. Elevated expression of Foxp3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner. *Clin Immunol* 2010; **134**: 277-288 [PMID: 19900843 DOI: 10.1016/j.clim.2009.10.005]

99 **Ahmadi M**, Emery DC, Morgan DJ. Prevention of both direct and cross-priming of antitumor CD8+ T-cell responses following overproduction of prostaglandin E2 by tumor cells in vivo. *Cancer Res* 2008; **68**: 7520-7529 [PMID: 18794140 DOI: 10.1158/0008-5472.can-08-1060]

100 **Chen TH**, Fukuhara K, Mandai M, Matsumura N, Kariya M, Takakura K, Fujii S. Increased cyclooxygenase-2 expression is correlated with suppressed antitumor immunity in cervical adenocarcinomas. *Int J Gynecol Cancer* 2006; **16**: 772-779 [PMID: 16681759 DOI: 10.1111/j.1525-1438.2006.00385.x]

P- Reviewers: Shen LZ, Wang HX **S- Editor**: Wen LL
L- Editor: Webster JR **E- Editor**: Ma S

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7369

World J Gastroenterol 2013 November 14; 19(42): 7369-7373
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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MINIREVIEWS

Intestinal acyl-CoA synthetase 5: Activation of long chain fatty acids and behind

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Received: June 21, 2013 Revised: July 20, 2013
Accepted: September 29, 2013
Published online: November 14, 2013

Abstract

The intestinal mucosa is characterized by a high complexity in terms of structure and functions and allows for a controlled demarcation towards the gut lumen. On the one hand it is responsible for pulping and selective absorption of alimentary substances ensuring the immunological tolerance, on the other hand it prevents the penetration of micro-organisms as well as bacterial outgrowth. The continuous regeneration of surface epithelia along the crypt-villus-axis in the small intestine is crucial to assuring these various functions. The core phenomena of intestinal epithelia regeneration comprise cell proliferation, migration, differentiation, and apoptosis. These partly contrarily oriented processes are molecularly balanced through numerous interacting signaling pathways like Wnt/β-catenin, Notch and Hedgehog, and regulated by various modifying factors. One of these modifiers is acyl-CoA synthetase 5 (ACSL5). It plays a key role in *de novo* lipid synthesis, fatty acid degradation and membrane modifications, and regulates several intestinal processes, primarily through different variants of protein lipidation, *e.g.*, palmitoylation. ACSL5 was shown to interact with proapoptotic molecules, and besides seems to inhibit proliferation along the crypt-villus-axis. Because of its proapoptotic and antiproliferative characteristics it could be of significant relevance for intestinal homeostasis, cellular disorder and tumor development.

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Key words: Acyl-CoA synthetase; Apoptosis; Carcinogenesis; Colorectal cancer; Intestinal homeostasis

Core tip: Acyl-CoA synthetase 5 (ACSL5) activates long-chain fatty acids by coenzyme A linkage and plays a key role in fatty acid metabolism. On the basis of its mitochondrial localization, ACSL5 forms an exceptional member among the acyl-CoA synthetase family. Although its various functions are not yet fully understood, ACSL5 seems to represent a modifier of cellular vitality along the crypt-villus-axis.

Klaus C, Jeon MK, Kaemmerer E, Gassler N. Intestinal acyl-CoA synthetase 5: Activation of long chain fatty acids and behind. *World J Gastroenterol* 2013; 19(42): 7369-7373 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7369.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7369

INTRODUCTION

Acyl-CoA synthetase 5 (ACSL5) is part of the acyl-CoA synthetase family who plays a key role in *de novo* lipid synthesis, fatty acid degradation and subsequently membrane modifications. Their main function is the activation of long-chain fatty acids by coenzyme A linkage, these conjugates are primarily utilized for triglyceride synthesis, packed into chylomicrons and secreted through the basolateral membrane[1-3]. Along the intestinal crypt-villus-axis (CVA) they either cause cell proliferation or cell apoptosis[4]. The fatty acid activation requires a two-step reaction, catalysed through these enzymes. The first step results in an acyl-AMP intermediate of ATP. AMP is then replaced with CoA and the activated acyl-CoA is generated. Except for the resulting acyl-CoA, free fatty acids (the substrates of these enzymes) are able to pass through membranes. The ACSL-derived esterification prevents it from leaving the cell. The length of the carbon chain (12-20 C atoms) of the fatty acids defines the substrate specifics of the different acyl-CoA synthetases. On this basis, five different subfamilies were identified[5]. Their sequences differ at N-terminus, which is presumably the reason for their diversity in sub-cellular localisation. ACSL1 and 6 are associated with the plasma membrane. They might play a role in the cellular fatty acid assimilation[6,7]. ACSL4 was mainly found in ER and peroxisomes[8]. The gene *ACSL5* lies on chromosome 10q25.1-q25.2[9,10]. The functional protein is localized on the inner mitochondrial membrane[11,12] and has a significant regulatory function in the mitochondrial energy metabolism. The pH-dependence of this molecule is especially important. It shows a high functional activity under alkaline conditions. This observation is of special interest in the case of mitochondria: In contrast to the enclosing cytoplasm their pH is more basic by one point and they show a more alkaline pH with beginning apoptosis[13]. In this context the pH conditions of tumor genesis and a differing functionality of ACSL5 should be considered as well[14].

Analysing the role of ACSL5 in differentiation and senescence of enterocytes, a sensitization to TRAIL-dependent apoptosis was found[4], whilst the Triacsin C-induced apoptosis induction in glioma cells was inhibited. Mashima *et al*[9] postulated an ACSL5-dependent survival of tumor cells. There is evidence to suggest a cell regulatory involvement of ACSL5 in addition to its lipid modifying function[15].

Triacsin C [1-hydroxy-3-(E,E,E-2',4',7'-undecatrienylidine) triazene] was identified as a potent competitive inhibitor of acyl-CoA synthetase activity[16]. Its inhibitory effects depend on the N-hydroxytriazene moiety of the molecule, resulting in a dramatic reduction in cholesterol as well as triglyceride synthesis with non-transition of macrophages to foam cells or enhanced eicosanoid release in leucocytes[17,18]. Kaemmerer *et al*[18] showed that human ACSL5 is, unlike rat ACSL5, sensitive to the competitive inhibition by triacsin C and does not compensate for other triacsin C sensitive ACSL isoforms.

This mini review outlines the modifying role of ACSL5 in different cellular processes at the interface between proliferation and apoptosis.

ACSL5 AS A REGULATOR OF THE CELLULAR RENEWAL ALONG THE CVA IN HUMAN SMALL INTESTINE

The CVA in adult human small intestine is important for maintaining intestinal homeostasis and this process is controlled by regulatory signaling pathways such as Wnt/β-catenin, Notch, Hedgehog, and apoptosis. Canonical Wnt signaling plays a key role in regulating intestinal cell proliferation[19]. A mutation-derived activation of the cascade initiates the adenocarcinoma sequence. Blocking canonical Wnt signals leads to a proliferative arrest of epithelial cells in the crypts of Lieberkuhn. The cell sorting receptors EphB2 and B3 are also target molecules of the pathway, as they control the positioning of Paneth cells towards the crypt bottom, whereas all other differentiated epithelial cells migrate along the CVA[20].

The highest amount of Notch activity is found in intestinal stem cells[21,22]. The transcription factors Hes-1 and Math-1 conduct the differentiation of progenitor cells into enteroendocrine, goblet and Paneth cells[23,24]. Blocking of Notch signaling with the γ-secretase inhibitor dibenzazepine results in a rapid conversion of proliferative crypt cells into post-mitotic goblet cells[25,26].

The Hedgehog pathway with its main epithelial proteins Sonic (Shh) and Indian (Ihh) Hedgehog is of central relevance in villus formation and stem cell homeostasis[27,28].

ACSL5 is expressed in an ascending gradient along the CVA with the highest expression level in enterocytes at the villus tip (Figure 1). It is assumed that ACSL5 sensitizes these enterocytes towards TRAIL-derived apoptosis susceptibility by down-regulation of the anti-apoptotic FLIP and up-regulation of TRAIL-R1 on the cell surface. TRAIL shows a corresponding gradient expression pattern[4]. Gassler *et al*[4] suggested an ACSL5-dependent link to TRAIL-induced apoptosis by the ceramide neosynthesis, which is known to play a key role in TRAIL-induced apoptosis[29], occurring mainly at mitochondrial membranes[30].

Adenocarcinomas with an invasive phenotype and enhanced proliferation showed decreased levels of ACSL5[4]. A functional correlation between ACSL5-derived apoptosis susceptibility of enterocytes and increased tumor development is assumed[31].

LIPID MODIFICATIONS CONTROL INTESTINAL SIGNALING PATHWAYS OF CENTRAL RELEVANCE

Long-chain polyunsaturated fatty acids show significant modifying functions in the cell cycle, resulting in inflammation and cancer development[32,33]. They enter into cells by means of concentration gradients or transport proteins. The retention in the cell depends on its further metabolic function and intracellular modification. The different ACSL isoforms activate a broad range of long-chain fatty acids and channel them into specific downstream pathways that can vary in a tissue-specific manner[34]. An over-expression of ACSL5 increases the fatty acid incorporation into diacylglycerol and triacylglycerol but does not affect FA used for β-oxidation[35].

A lipid modification of central relevance in several intestinal pathways is palmitoylation. Palmitate, a 16-carbon saturated fatty acid, is covalently bound to more than 100

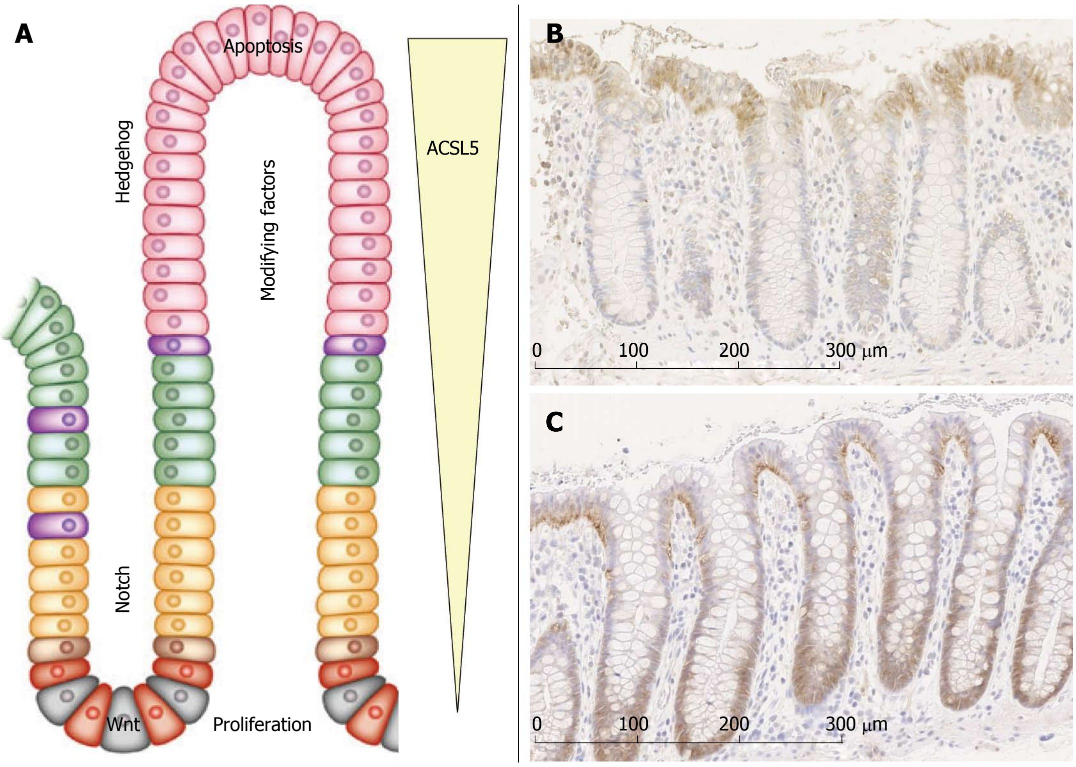

Figure 1 Expression of acyl-CoA synthetase 5 and β-catenin in normal human intestinal mucosa. Schematic overview of signaling pathways in intestinal mucosa (A) immunostaining of acyl-CoA synthetase 5 (ACSL5) (B) and β-catenin (C).

cellular and viral proteins. In most cases, the fatty acid is thioester (S-acyl) posttranslationally linked to cysteine. The reaction is catalysed by palmitoyl-acyltransferases, where palmitoyl-CoA serves as the donor. The palmitoylation is a dynamic process by means of its reversible linkage[36-38]. Palmitoylation has several effects on molecule functionality and is especially relevant for signaling cascades[39,40]. Protein hydrophobicity and membrane association are increased and protein/protein or protein/lipid interactions are augmented[41]. Modifications derived from fatty acids are described for the proteins associated with Wnt signaling, which are very important for secretion and activity. A fatty acid modification at serine promotes cysteine palmitoylation[42,43]. Without this fatty acid attachment at the Wnt ligand, *N*-glycosylation and secretion were still possible. In contrast, the absence of *N*-glycosylation resulted in a decreased secretion rate.

One example for an apoptosis-associated ligand that is regulated by palmitoylation is the human Fas ligand (FasL), responsible for correct signal transduction finally resulting in cell death. Palmitoylation regulates its transmembrane domain and is critical for efficient FasL-mediated killing and FasL processing[44,45].

Another molecule regulated by *S*-palmitoylation is γ-secretase. Its diverse substrates originate from several pathways with a focus on type Ⅰ integral membrane proteins, including Notch1 homologues, Notch ligands, Delta and Jagged, cell adhesion receptors N- and E-cadherins, low density lipoprotein receptor-related protein, ErbB-4, netrin receptor DCC[46,47]. Cheng *et al*[46] identified S-palmitoylation of γ-secretase subunits nicastrin and APH-1, and characterized its role on DRM association, protein stability, and γ-secretase enzyme activities.

Palmitoylation of Hedgehog proteins is important for effective signaling. Protein fatty acylation is not only found to be a regulator for membrane association of intracellular proteins, but also for the signaling activity and efficacy of secreted proteins[48,49]. Palmitoylation thus controls mechanisms leading to an altered reaction of the cells towards external signaling.

ACSL5 may play a role in channelling fatty acids towards palmitoylation and other lipid functions with high relevance for cellular behaviour[18]. An over-expression of ACSL5 in HepG2 showed an increased palmitate oxidation[50]. An ACSL5-mediated lipid modification of proliferation- and apoptosis-associated molecules might be an interface in the intestinal CVA.

CONCLUSION

The intestinal epithelium is subject to continuous self-renewal along the CVA, and progress has been made towards understanding the task of the various signal transduction pathways in proliferation, differentiation, migration and apoptosis of epithelial enterocytes. ACSL5 is a protein localized in mitochondria that plays a key role in fatty acid metabolism and apoptosis. Although its various functions are not yet fully understood, ACSL5 seems to represent a modifier of cellular vitality along the CVA, providing fatty acids for several molecular events.

REFERENCES

1 **Meller N**, Morgan ME, Wong WP, Altemus JB, Sehayek E. Targeting of Acyl-CoA synthetase 5 decreases jejunal fatty acid activation with no effect on dietary long-chain fatty acid absorption. *Lipids Health Dis* 2013; **12**: 88 [PMID: 23767941 DOI: 10.1186/1476-511X-12-88]

2 **Kindel T**, Lee DM, Tso P. The mechanism of the formation and secretion of chylomicrons. *Atheroscler Suppl* 2010; **11**: 11-16 [PMID: 20493784 DOI: 10.1016/j.atherosclerosissup.2010.03.003]

3 **Watkins PA**, Maiguel D, Jia Z, Pevsner J. Evidence for 26 distinct acyl-coenzyme A synthetase genes in the human genome. *J Lipid Res* 2007; **48**: 2736-2750 [PMID: 17762044]

4 **Gassler N**, Roth W, Funke B, Schneider A, Herzog F, Tischendorf JJ, Grund K, Penzel R, Bravo IG, Mariadason J, Ehemann V, Sykora J, Haas TL, Walczak H, Ganten T, Zentgraf H, Erb P, Alonso A, Autschbach F, Schirmacher P, Knüchel R, Kopitz J. Regulation of enterocyte apoptosis by acyl-CoA synthetase 5 splicing. *Gastroenterology* 2007; **133**: 587-598 [PMID: 17681178]

5 **Soupene E**, Kuypers FA. Multiple erythroid isoforms of human long-chain acyl-CoA synthetases are produced by switch of the fatty acid gate domains. *BMC Mol Biol* 2006; **7**: 21 [PMID: 16834775]

6 **Ellis JM**, Mentock SM, Depetrillo MA, Koves TR, Sen S, Watkins SM, Muoio DM, Cline GW, Taegtmeyer H, Shulman GI, Willis MS, Coleman RA. Mouse cardiac acyl coenzyme a synthetase 1 deficiency impairs Fatty Acid oxidation and induces cardiac hypertrophy. *Mol Cell Biol* 2011; **31**: 1252-1262 [PMID: 21245374 DOI: 10.1128/MCB.01085-10]

7 **Li LO**, Ellis JM, Paich HA, Wang S, Gong N, Altshuller G, Thresher RJ, Koves TR, Watkins SM, Muoio DM, Cline GW, Shulman GI, Coleman RA. Liver-specific loss of long chain acyl-CoA synthetase-1 decreases triacylglycerol synthesis and beta-oxidation and alters phospholipid fatty acid composition. *J Biol Chem* 2009; **284**: 27816-27826 [PMID: 19648649 DOI: 10.1074/jbc.M109.022467]

8 **Brasaemle DL**, Dolios G, Shapiro L, Wang R. Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes. *J Biol Chem* 2004; **279**: 46835-46842 [PMID: 15337753]

9 **Mashima T**, Sato S, Okabe S, Miyata S, Matsuura M, Sugimoto Y, Tsuruo T, Seimiya H. Acyl-CoA synthetase as a cancer survival factor: its inhibition enhances the efficacy of etoposide. *Cancer Sci* 2009; **100**: 1556-1562 [PMID: 19459852 DOI: 10.1111/j.1349-7006.2009.01203.x]

10 **Yamashita Y**, Kumabe T, Cho YY, Watanabe M, Kawagishi J, Yoshimoto T, Fujino T, Kang MJ, Yamamoto TT. Fatty acid induced glioma cell growth is mediated by the acyl-CoA synthetase 5 gene located on chromosome 10q25.1-q25.2, a region frequently deleted in malignant gliomas. *Oncogene* 2000; **19**: 5919-5925 [PMID: 11127823]

11 **Lewin TM**, Granger DA, Kim JH, Coleman RA. Regulation of mitochondrial sn-glycerol-3-phosphate acyltransferase activity: response to feeding status is unique in various rat tissues and is discordant with protein expression. *Arch Biochem Biophys* 2001; **396**: 119-127 [PMID: 11716470]

12 **Coleman RA**, Lewin TM, Van Horn CG, Gonzalez-Baró MR. Do long-chain acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? *J Nutr* 2002; **132**: 2123-2126 [PMID: 12163649]

13 **Matsuyama S**, Reed JC. Mitochondria-dependent apoptosis and cellular pH regulation. *Cell Death Differ* 2000; **7**: 1155-1165 [PMID: 11175252]

14 **Mashima T**, Oh-hara T, Sato S, Mochizuki M, Sugimoto Y, Yamazaki K, Hamada J, Tada M, Moriuchi T, Ishikawa Y, Kato Y, Tomoda H, Yamori T, Tsuruo T. p53-defective tumors with a functional apoptosome-mediated pathway: a new therapeutic target. *J Natl Cancer Inst* 2005; **97**: 765-777 [PMID: 15900046]

15 **Reinartz A**, Ehling J, Leue A, Liedtke C, Schneider U, Kopitz J, Weiss T, Hellerbrand C, Weiskirchen R, Knüchel R, Gassler N. Lipid-induced up-regulation of human acyl-CoA synthetase 5 promotes hepatocellular apoptosis. *Biochim Biophys Acta* 2010; **1801**: 1025-1035 [PMID: 20470896 DOI: 10.1016/j.bbalip.2010.04.010]

16 **Tomoda H**, Igarashi K, Cyong JC, Omura S. Evidence for an essential role of long chain acyl-CoA synthetase in animal cell proliferation. Inhibition of long chain acyl-CoA synthetase by triacsins caused inhibition of Raji cell proliferation. *J Biol Chem* 1991; **266**: 4214-4219 [PMID: 1999415]

17 **Namatame I**, Tomoda H, Arai H, Inoue K, Omura S. Complete inhibition of mouse macrophage-derived foam cell formation by triacsin C. *J Biochem* 1999; **125**: 319-327 [PMID: 9990129]

18 **Kaemmerer E**, Peuscher A, Reinartz A, Liedtke C, Weiskirchen R, Kopitz J, Gassler N. Human intestinal acyl-CoA synthetase 5 is sensitive to the inhibitor triacsin C. *World J Gastroenterol* 2011; **17**: 4883-4889 [PMID: 22171129 DOI: 10.3748/wjg.v17.i44.4883]

19 **Cordero JB**, Sansom OJ. Wnt signalling and its role in stem cell-driven intestinal regeneration and hyperplasia. *Acta Physiol (Oxf)* 2012; **204**: 137-143 [PMID: 21439026 DOI: 10.1111/j.1748-1716.2011.02288.x]

20 **Ahuja V**, Dieckgraefe BK, Anant S. Molecular biology of the small intestine. *Curr Opin Gastroenterol* 2006; **22**: 90-94 [PMID: 16462162]

21 **Radtke F**, Clevers H. Self-renewal and cancer of the gut: two sides of a coin. *Science* 2005; **307**: 1904-1909 [PMID: 15790842]

22 **Perdigoto CN**, Bardin AJ. Sending the right signal: Notch and stem cells. *Biochim Biophys Acta* 2013; **1830**: 2307-2322 [PMID: 22917651 DOI: 10.1016/j.bbagen.2012.08.009]

23 **Schröder N**, Gossler A. Expression of Notch pathway components in fetal and adult mouse small intestine. *Gene Expr Patterns* 2002; **2**: 247-250 [PMID: 12617809]

24 **Kim TH**, Shivdasani RA. Genetic evidence that intestinal Notch functions vary regionally and operate through a common mechanism of Math1 repression. *J Biol Chem* 2011; **286**: 11427-11433 [PMID: 21282114 DOI: 10.1074/jbc.M110.188797]

25 **van Es JH**, van Gijn ME, Riccio O, van den Born M, Vooijs M, Begthel H, Cozijnsen M, Robine S, Winton DJ, Radtke F, Clevers H. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* 2005; **435**: 959-963 [PMID: 15959515]

26 **Bu X**, Li N, Tian X, Huang P. Gamma-secretase inhibitor, a potential target therapy for MUC2-positive colorectal carcinoma. *Neoplasma* 2011; **58**: 343-347 [PMID: 21520992]

27 **Watt FM**. Unexpected Hedgehog-Wnt interactions in epithelial differentiation. *Trends Mol Med* 2004; **10**: 577-580 [PMID: 15567325]

28 **Jones RG**, Li X, Gray PD, Kuang J, Clayton F, Samowitz WS, Madison BB, Gumucio DL, Kuwada SK. Conditional deletion of beta1 integrins in the intestinal epithelium causes a loss of Hedgehog expression, intestinal hyperplasia, and early postnatal lethality. *J Cell Biol* 2006; **175**: 505-514 [PMID: 17088430]

29 **Lee YJ**, Amoscato AA. TRAIL and ceramide. *Vitam Horm* 2004; **67**: 229-255 [PMID: 15110180]

30 **Siskind LJ**, Kolesnick RN, Colombini M. Ceramide forms channels in mitochondrial outer membranes at physiologically relevant concentrations. *Mitochondrion* 2006; **6**: 118-125 [PMID: 16713754]

31 **Gassler N**, Schneider A, Kopitz J, Schnölzer M, Obermüller N, Kartenbeck J, Otto HF, Autschbach F. Impaired expression of acyl-CoA-synthetase 5 in epithelial tumors of the small intestine. *Hum Pathol* 2003; **34**: 1048-1052 [PMID: 14608540]

32 **Chapkin RS**, McMurray DN, Davidson LA, Patil BS, Fan YY, Lupton JR. Bioactive dietary long-chain fatty acids: emerging

mechanisms of action. *Br J Nutr* 2008; **100**: 1152-1157 [PMID: 18492298 DOI: 10.1017/S0007114508992576]

33 **Digel M**, Ehehalt R, Stremmel W, Füllekrug J. Acyl-CoA synthetases: fatty acid uptake and metabolic channeling. *Mol Cell Biochem* 2009; **326**: 23-28 [PMID: 19115050 DOI: 10.1007/s11010-008-0003-3]

34 **Mashek DG**, Coleman RA. Cellular fatty acid uptake: the contribution of metabolism. *Curr Opin Lipidol* 2006; **17**: 274-278 [PMID: 16680032]

35 **Ellis JM**, Frahm JL, Li LO, Coleman RA. Acyl-coenzyme A synthetases in metabolic control. *Curr Opin Lipidol* 2010; **21**: 212-217 [PMID: 20480548]

36 **Resh MD**. Palmitoylation of ligands, receptors, and intracellular signaling molecules. *Sci STKE* 2006; **2006**: re14 [PMID: 17077383]

37 **Mitchell DA**, Vasudevan A, Linder ME, Deschenes RJ. Protein palmitoylation by a family of DHHC protein S-acyltransferases. *J Lipid Res* 2006; **47**: 1118-1127 [PMID: 16582420]

38 **Conibear E**, Davis NG. Palmitoylation and depalmitoylation dynamics at a glance. *J Cell Sci* 2010; **123**: 4007-4010 [PMID: 21084560 DOI: 10.1242/jcs.059287]

39 **Smotrys JE**, Linder ME. Palmitoylation of intracellular signaling proteins: regulation and function. *Annu Rev Biochem* 2004; **73**: 559-587 [PMID: 15189153]

40 **Aicart-Ramos C**, Valero RA, Rodriguez-Crespo I. Protein palmitoylation and subcellular trafficking. *Biochim Biophys Acta* 2011; **1808**: 2981-2994 [PMID: 21819967 DOI: 10.1016/j.bbamem.2011.07.009]

41 **Simons K**, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000; **1**: 31-39 [PMID: 11413487]

42 **Doubravska L**, Krausova M, Gradl D, Vojtechova M, Tumova L, Lukas J, Valenta T, Pospichalova V, Fafilek B, Plachy J, Sebesta O, Korinek V. Fatty acid modification of Wnt1 and Wnt3a at serine is prerequisite for lipidation at cysteine and is essential for Wnt signalling. *Cell Signal* 2011; **23**: 837-848 [PMID: 21244856 DOI: 10.1016/j.cellsig.2011.01.007]

43 **Martin BR**, Wang C, Adibekian A, Tully SE, Cravatt BF. Global profiling of dynamic protein palmitoylation. *Nat Methods* 2012; **9**: 84-89 [PMID: 22056678 DOI: 10.1038/nmeth.1769]

44 **Guardiola-Serrano F**, Rossin A, Cahuzac N, Lückerath K, Melzer I, Mailfert S, Marguet D, Zörnig M, Hueber AO. Palmitoylation of human FasL modulates its cell death-inducing function. *Cell Death Dis* 2010; **1**: e88 [PMID: 21368861 DOI: 10.1038/cddis.2010.62]

45 **Chakrabandhu K**, Hérincs Z, Huault S, Dost B, Peng L, Conchonaud F, Marguet D, He HT, Hueber AO. Palmitoylation is required for efficient Fas cell death signaling. *EMBO J* 2007; **26**: 209-220 [PMID: 17159908]

46 **Cheng H**, Vetrivel KS, Drisdel RC, Meckler X, Gong P, Leem JY, Li T, Carter M, Chen Y, Nguyen P, Iwatsubo T, Tomita T, Wong PC, Green WN, Kounnas MZ, Thinakaran G. S-palmitoylation of gamma-secretase subunits nicastrin and APH-1. *J Biol Chem* 2009; **284**: 1373-1384 [PMID: 19028695 DOI: 10.1074/jbc.M806380200]

47 **Vetrivel KS**, Zhang YW, Xu H, Thinakaran G. Pathological and physiological functions of presenilins. *Mol Neurodegener* 2006; **1**: 4 [PMID: 16930451]

48 **Buglino JA**, Resh MD. Palmitoylation of Hedgehog proteins. *Vitam Horm* 2012; **88**: 229-252 [PMID: 22391306 DOI: 10.1016/B978-0-12-394622-5.00010-9]

49 **Pirola L**, Zerzaihi O, Vidal H, Solari F. Protein acetylation mechanisms in the regulation of insulin and insulin-like growth factor 1 signalling. *Mol Cell Endocrinol* 2012; **362**: 1-10 [PMID: 22683437 DOI: 10.1016/j.mce.2012.05.011]

50 **Zhou Y**, Abidi P, Kim A, Chen W, Huang TT, Kraemer FB, Liu J. Transcriptional activation of hepatic ACSL3 and ACSL5 by oncostatin m reduces hypertriglyceridemia through enhanced beta-oxidation. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2198-2205 [PMID: 17761945]

P- Reviewers: Handa O, Jeung EB, Mattner J **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Zhang DN

WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7374

World J Gastroenterol 2013 November 14; 19(42): 7374-7388
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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ORIGINAL ARTICLE

1,3-Bis(2-chloroethyl)-1-nitrosourea enhances the inhibitory effect of Resveratrol on 5-fluorouracil sensitive/resistant colon cancer cells

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Supported by Indian Council of Medical Research and Department of Biotechnology, Government of India

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Received: December 19, 2012 **Revised:** April 25, 2013
Accepted: June 5, 2013
Published online: November 14, 2013

Abstract

AIM: To study the mechanism of 5-fluorouracil (5-FU) resistance in colon cancer cells and to develop strategies for overcoming such resistance by combination treatment.

METHODS: We established and characterized a 5-FU resistance (5-FU-R) cell line derived from continuous exposure (25 μmol/L) to 5-FU for 20 wk in 5-FU sensitive HCT-116 cells. The proliferation and expression of different representative apoptosis and anti-apoptosis markers in 5-FU sensitive and 5-FU resistance cells were measured by the MTT assay and by Western blotting, respectively, after treatment with Resveratrol (Res) and/or 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU). Apoptosis and cell cycle arrest was measured by 4',6'-diamidino-2-phenylindole hydrochloride staining and fluorescence-activated cell sorting analysis, respectively. The extent of DNA damage was measured by the Comet assay. We measured the visible changes in the DNA damage/repair cascade by Western blotting.

RESULTS: The widely used chemotherapeutic agents BCNU and Res decreased the growth of 5-FU sensitive HCT-116 cells in a dose dependent manner. Combined application of BCNU and Res caused more apoptosis in 5-FU sensitive cells in comparison to individual treatment. In addition, the combined application of BCNU and Res caused a significant decrease of major DNA base excision repair components in 5-FU sensitive cells. We established a 5-FU resistance cell line (5-FU-R) from 5-FU-sensitive HCT-116 (mismatch repair deficient) cells that was not resistant to other chemotherapeutic agents (*e.g.*, BCNU, Res) except 5-FU. The 5-FU resistance of 5-FU-R cells was assessed by exposure to increasing concentrations of 5-FU followed by the MTT assay. There was no significant cell death noted in 5-FU-R cells in comparison to 5-FU sensitive cells after 5-FU treatment. This resistant cell line overexpressed anti-apoptotic [*e.g.*, AKT, nuclear factor κB, FLICE-like inhibitory protein), DNA repair (*e.g.*, DNA polymerase beta (POL-β), DNA polymerase eta (POLH), protein Flap endonuclease 1 (FEN1), DNA damage-binding protein 2 (DDB2)] and 5-FU-resistance proteins (thymidylate synthase) but under expressed pro-apoptotic proteins (*e.g.*, DAB2, CK1) in comparison to the parental cells. Increased genotoxicity and apoptosis were observed in resistant cells after combined application of BCNU and Res in comparison to untreated or parental cells. BCNU increased the sensitivity to Res of 5-FU resistant cells compared with parental cells. Fifty percent cell death were noted in parental cells when 18 μmol/L of Res was associated with fixed concentration (20 μmol/L) of BCNU, but a much lower concentration of Res (8 μmol/L) was needed to achieve the same effect in 5-FU resistant cells. Interestingly, increased levels of adenomatous polyposis coli and decreased levels POL-β, POLH, FEN1 and DDB2 were noted after the same combined treatment in resistant cells.

CONCLUSION: BCNU combined with Res exerts a synergistic effect that may prove useful for the treatment of colon cancer and to overcome drug resistance.

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Key words: 5-fluorouracil; 1,3-Bis(2-chloroethyl)-1-nitrosourea; Resveratrol; Colon cancer; Combination therapy

Core tip: 5-fluorouracil (5-FU) resistance in colon cancer patients is a common phenomenon that requires immediate resolution. In this paper, we used two commonly administered clinical drugs, 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and Resveratrol (Res), and studied their effects on 5-FU sensitive and resistance colon cancer cells. The drug combination was more effective in 5-FU resistant cells than in 5-FU sensitive cells and was more effective than the individual treatments. The BCNU-Res combination mediated its action by compromising the base excision repair cascade. This combination might be useful for treatment of 5-FU resistant colon cancer patients.

Das D, Preet R, Mohapatra P, Satapathy SR, Kundu CN. 1,3-Bis(2-chloroethyl)-1-nitrosourea enhances the inhibitory effect of Resveratrol on 5-fluorouracil sensitive/resistant colon cancer cells. *World J Gastroenterol* 2013; 19(42): 7374-7388 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7374.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7374

INTRODUCTION

Colon cancer ranks as second in cancer related deaths in Western countries, and accounts for approximately 10%-15% of all cancers; half of these patients eventually metastasize[1]. The prognosis for patients who develop metastatic tumors is very poor, although several chemotherapeutic methods have been used to improve their survival and quality of life[2]. The antimetabolite 5-fluorouracil (5-FU) has been used as a first line therapy in colon cancer; however, in the clinical setting, more than 40% of cases are resistant. Combinations of 5-FU with Leucovorin (LV), Cisplatin, and Oxaloplatin, or with plant derived compounds including Curcumin, Resveratrol, and Quinacrine have increased the response rate up to 25%-30%[3-8]. 5-FU exerts anti-proliferative and cytotoxic effects on cells by inhibiting thymidylate synthase (TS) or by misincorporation into DNA and RNA[9].

The misincorporation of 5-fluorodeoxyribosyl residues in DNA is generally repaired by mismatch repair (MMR)[10-12] and cells grow continuously. However, any disturbance of the repair system will cause incomplete removal of 5-FU, leading to cell death. Thus, resistance to 5-FU in certain cancers may be the result of highly efficient DNA repair in the 5-FU resistant cells. Several investigators have used multiple combinations of drugs to increase the sensitivity of 5-FU resistant-cancer cells, but these studies showed very poor clinical responses[4-6,8].

The well-known natural product Resveratrol (Res) has demonstrated high antitumoral efficiency without harmful side effects of conventional chemotherapies. Res (3,4,5-trihydroxy-trans-stilbene) is a polyphenolic phytoalexin widely present in plants and enriched in red grapes, peanuts and other sources[13]. This compound demonstrates beneficial functions in normal cells both in *in vitro* and *in vivo* model systems[13]. On the other hand, this compound exhibits cytotoxic effects on the majority of malignant cells, blocking the three major stages of carcinogenesis (*i.e.*, initiation, promotion and progression)[13] in several cancer cell types, such as breast, colon, melanoma, uterine, lung and leukemia cells[13-21]. Recently, using HCT-116 colon cancer cells, we demonstrated that 5-FU increases the sensitivity to resveratrol by inducing DNA damage and the MAPK pathway[7]. A significant inhibition of cell proliferation, migration, and increased apoptosis were observed when moderate concentration of Res (15 μmol/L) were combined with very low concentration of 5-FU (0.5 μmol/L)[7].

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU or carmustine) belongs to the family of haloethylnitrosoureas. It is a common synthetic alkylating chemotherapeutic agent used for treating various types of cancers, including brain tumors, Hodgkins and non-Hodgkins lymphoma and multiple myeloma[22]. It is highly lipophilic, capable of readily crossing the blood-brain barrier. It reacts with DNA and forms several exocyclic DNA adducts, which includes the saturated ethanol adducts of adenine, cytosine, and guanine (1,*N*6-ethanol-A, 3,*N*4-ethanol-C, *N*2,3-ethanol-G, and 1,*O*6-ethanol-G) and produces intra strand cross-links in DNA[23-26]. It can also produce mono substituted purine bases in DNA[23]. BCNU is classified as an animal carcinogen[27], and has been demonstrated as genotoxic both *in vivo* and *in vitro*[27]. This limits the use of this drug at higher doses, unless the drug is combined with some natural anticancer compounds that will not only decrease the toxic effect, but also synergistically increase its effectiveness. Several combinations of BCNU have been tried, such as with As_2O_3[28], O^6BG[29] and TMZ[30], to increase the sensitivity in cancer cells, but without success.

Recently, researchers attempted to target DNA damage/repair and cell cycle checkpoint signaling pathways as a means of cancer treatment[31-33]. This is relevant for 5-FU therapy, as TS inhibition and incorporation of the fluorinated base into DNA occurs during S-phase. The PI3K-like kinases, ataxia telangiectasia-mutated (ATM) and ATM-related (ATR), are central mediators in the response to DNA damage during S-phase[34]. Evidence suggests the involvement of S-phase checkpoint pathways in response to 5-FU treatment and TS inhibition[35]. A recent report suggested that ATR and CHK1 status influence cellular sensitivity to 5-FU in a MMR- or BER-mediated response-dependent manner, dictated by the drug dose and exposure period[36]. It was also reported that 5-FU-mediated apoptosis also involved modulation of the major long patch base excision repair (LP-BER) protein Flap endonuclease 1 (FEN1)[10,37]. We have already reported that activation of adenomatous polyposis coli (APC) blocks the base excision repair pathway by interaction with DNA polymerase beta (POL-β) and FEN1[38-41]. Thus, one strategy for killing 5-FU resistant cells includes combining multiple drugs that will damage DNA, inhibit the DNA repair system and hamper cell cycle regulation in cancer cells.

In the present study, we developed a novel chemotherapeutic combination to increase the sensitivity of 5-FU resistant colon cancer cells using a synthetic DNA damaging agent, BCNU, and a plant derived anti-cancer agent, Res, and investigated the mechanism of anti cancer potentiality against 5-FU resistance. We first measured the anti-cancer potentiality of this combination in 5-FU sensitive human colon cancer cells. A 5-FU resistant cell line was derived from HCT-116 colon cancer cells after prolonged treatment with 5-FU, which was sensitive to other chemotherapeutic agents. These cells were treated with the BCNU and Res combination and drug efficacy was measured. The BCNU and Res combination increased apoptosis of the 5-FU resistant cell lines by inducing DNA damage and inhibiting the base excision repair pathway. We also used isobologram analysis to evaluate whether the drug combination has a synergistic, additive or antagonist effect on human colon cancer.

MATERIALS AND METHODS

Cell culture and treatment

The HCT-116 colon cancer cell line (obtained from American Type Culture Collection, VA, United States, Cat # CCL-247) was cultured in RPMI-1640 with 1% antibiotic (100 units of penicillin and 1 mg streptomycin per milliliter in 0.9% normal saline) and supplemented with 10% fetal bovine serum (HIMEDIA, Mumbai, India) in a humidified CO_2 incubator in 5% CO_2 at 37 ℃. Drugs like Res, 5-FU, LV, BCNU were purchased from Sigma Chemical Ltd. (St. Louis, MO, United States). DNA polymerase eta (POLH) and DNA damage-binding protein 2 (DDB2) antibodies were procured from Abcam (MA, United States). Apurinic/apyrimidinic (AP) endonuclease 1 (APE) and POL-β antibodies were purchased from Novus Biologicals (CO, United States). The anti-APC antibody was purchased from Calbiochem (CA, United States). All the other antibodies used in the experiments were procured from Cell Signaling Technology (Danvers, MA, United States). A 1 mmol/L stock of Res, 5-FU, BCNU and LV was prepared in absolute ethanol, DMSO, 50% ethanol and distilled water, respectively, and was stored at -20 ℃. During treatment, Res, 5-FU and BCNU were diluted in RPMI and then added to the cultures to achieve the desired final concentration. LV (1 μmol/L) was added in combination with 5-FU in each treatment. When they reached 60%-70% confluency, the cells were treated with 5-FU, Res, BCNU and the combination of BCNU and Res (in each case of the combination treatment, the cells were pre-treated with BCNU (20 μmol/L) for 24 h and then exposed to Res for another 48 h).

Establishment of 5-FU resistant cell line

In our earlier report, we demonstrated that the minimum concentrations needed for causing fifty percent cell death in culture (LC_{50}) in HCT-116 cells by 5-FU was 10 μmol/L[7]. To make stable 5-FU resistant cell lines, HCT-116 cells were exposed to 25 μmol/L for more than 20 wk. Every 48 h the media was replaced with fresh media and cells were treated with fresh 5-FU of same concentration. Initially, more than 80% of the cells died within a few days; the remaining 20% cells survived and developed 5-FU resistance. At the end of the exposure, 5-FU resistance was checked by a cell survival assay (MTT) in the presence of 5-FU and the transformed characteristic was confirmed by measuring several biomarkers. No significant cell death was noted after 5-FU treatment allowing the cells to be designated as 5-FU-R cells.

MTT assay

The anchorage dependent cell viability of normal HCT-116 and 5-FU-R cells was measured after treatment with various drugs using a reliable and sensitive colorimetric assay: the MTT [3-(4,5-Dimethylthiazol-2yl-)-2,5-diphenyl tetrazolium bromide] cell proliferation assay, as described previously[7,42]. Approximately, 10000-12000 cells were plated in a 96 well tissue culture plate in triplicate and incubated for 24 h, after which they were exposed to various concentrations of the indicated compounds for 48 h. At the end of the treatment, the cells were washed with 1 × phosphate buffered saline (PBS) and then 100 μL of 0.05% MTT reagent (Sigma) was added to each well and kept for 6 h in incubation at 37 ℃ to allow the formation of purple formazan crystals. 100 μL of detergent solution (10% NP-40 with 4 mmol/L HCl) was added to each well of the 96 well tissue culture plate and the reaction mixture was incubated for 1hr at room temperature. The color intensity was measured spectrophotometrically using a microplate reader (Berthold, Germany) at 570 nm. The data were calculated as percentages of the control. All assays were performed at least three times.

Nuclear staining with 4’,6-diamidino-2-phenylindole

To determine the apoptosis of HCT-116 and 5-FU-R cells after treatment with the indicated drug, 4’,6-diamidino-2-phenylindole (DAPI) nuclear staining was carried out. Cells at 70%-80% confluency were treated with the desired drug for indicated time, as mentioned in the figure legend. The cells were then washed with PBS and fixed with acetone: methanol (1:1) for 15 min at -20 ℃ in the dark. Fixed cells were washed once with PBS and then DAPI solution was added and incubation was continued for 1 h at 37 ℃ in the dark. Excess DAPI was removed by washing with PBS and the stained cells were visualized under a fluorescence microscope (Nikon, Japan) at × 40 magnification.

Cell cycle

Regulation of cell cycle and apoptosis were measured using fluorescence-activated cell sorter (FACS) analysis. HCT-116 and 5-FU-R cells were cultured in 60 mm tissue culture discs until they reached 60%-70% confluency. They were then treated with the indicated drug. After treatment, cells were trypsinized and washed with PBS containing RNase-A and fixed with 70% ethanol. The cells were incubated at -20 ℃ overnight and then re-suspended in 0.1 mL of Propidium Iodide (PI, 50 μg/mL) and incubated for 1 h in the dark at room temperature. Finally, FACS was used to sort the cells (Becton and Dickinson, CA). Cell Quest Software was used to determine the DNA content of the cells at various phases of the cell cycle (Becton and Dickinson, CA).

Single cell gel electrophoresis or comet assay

After drug treatment, DNA damage in HCT-116 and 5-FU-R cells was determined by performing a single cell gel electrophoresis assay following the method of Tice *et al*[43]. Cells were suspended in PBS and about 5000-6000 cells were mixed with low melting agarose at 37 ℃ and spread on a microscopic slide. After solidifying, the slides were dipped in chilled lysis solution (10 mmol/L Tris, 100 mmol/L EDTA, 2.5 mol/L NaCl, 1% Triton × 100, 10% DMSO and pH 10.0) for 1 h. After lysis the slides were put in freshly prepared alkaline electrophoresis buffer and electrophoresis was performed at 20 V for 20 min. Slides were then dipped in neutralization buffer (0.4 mol/L Tris-HCl and pH 7.5) and washed with distilled water, followed by 70% ethanol, and allowed to dry. Later, 40 μL of SYBR green dye was added to each slide, which was incubated in the dark for 30 min at room temperature. A fluorescence microscope (Nikon, Japan) at a magnification of × 10 was used to visualize DNA migration. TriTek CometScore™ software was used to analyze the comet lengths.

Western blotting

Cells were plated on 100 mm tissue culture discs and were treated with the indicated drug when they reached 70% confluence. After treatment, the cells were washed with PBS and were lysed using the RIPA lysis buffer (50 mmol/L Tris, 150 mmol/L NaCl, 0.5 mmol/L Deoxycholate, 1% NP-40, 0.1% SDS, 1 mmol/L Na_3VO_4, 5 mmol/L EDTA, 1 mmol/L PMSF, 2 mmol/L DTT, 10 mmol/L β-glycerophosphate, 50 mmol/L NaF, 0.5% Triton × 100, protease inhibitor cocktail) for 45 min at 4 ℃. The lysates were centrifuged at 14000 rpm for 10 min to remove the cell debris. The supernatant was collected in a separate tube and protein quantification was performed by Bradford method using BSA as a standard. Approximately 100 μg of protein were loaded in each well and separated using 10% SDS-PAGE. After separation, the proteins were transferred to a PVDF membrane. The membrane was blocked and then probed with specific antibody, according to the manufacturer's protocol.

Analysis of combined drug effect

Synergistic, additive or antagonist drug effects were determined by isobologram analysis[28]. Isobologram plots were drawn by plotting the individual LC_{50} values of the drugs in their respective X- and Y-axis. The LC_{50} values were obtained from the individual drugs effect of MTT assays. Then, a line was used to join both the data points and the LC_{50} value of the combined drug was spotted on the same plot. In principle, if the spotted point (LC_{50} value of combined drugs) falls on the line then it considered as additive, whereas if it falls below or above the line, then it considered as a synergistic or antagonist drug effect, respectively.

RESULTS

Cytotoxic effect of 5-FU, BCNU and Res in HCT-116 colon cancer cells

To determine the LC_{50} (concentrations needed for fifty percent cell death in culture) and to understand the cytotoxic effect of each drug on HCT-116 cells, the MTT assay was carried out. Three known chemotherapeutic agents (5-FU, BCNU and Res) were chosen for experimentation (Figure 1A). The cells were treated for 48 h before the MTT assay. Figure 1A shows that increasing the concentrations of each drug increased cell death. 5-FU was the most effective among the three drugs and BCNU was the least effective. LC_{50} was noted at 10 μmol/L 5-FU while 35 μmol/L Res and 60 μmol/L BCNU were needed to achieve the same amount of cell death (Figure 1A). Although 5-FU offered maximum cytotoxic effects on colon cancer cells, more than 50% of cells exhibited resistance[44]. To overcome such resistance and to increase the efficacy of Res, a combined treatment was employed comprising BCNU and Res. The BCNU concentration was kept constant at 20 μmol/L and varied concentrations of Res were added. The combination of BCNU with Res increased the sensitivity of HCT-116 cells. Fifty percent cell death was observed when 18 μmol/L Res was combined with 20 μmol/L BCNU (Figure 1B) ($P < 0.05$).

Detection of apoptosis in HCT-116 cells by DAPI staining

5-FU, BCNU and Res killed colon cancer cells, but to confirm whether the killing effect of the drugs was through apoptosis or necrosis, DAPI nuclear staining was performed. Figure 1C shows that the number of apoptotic nuclei increased with increasing concentrations of Res when combined with 20 μmol/L BCNU as compared with untreated cells. More than fifty percent apoptotic nuclei were observed when 20 μmol/L BCNU was associated with 15 μmol/L of Res. Figure 1D shows a graphical representation of the number of apoptotic and non-apoptotic nuclei ($P < 0.05$).

Combined effect of BCNU and Res caused DNA damage in HCT-116 cells

To determine whether a combination of BCNU and Res caused apoptosis through DNA damage or by another mechanism, we measured the DNA damaging efficiency of this combination in HCT-116 cells by DNA damage

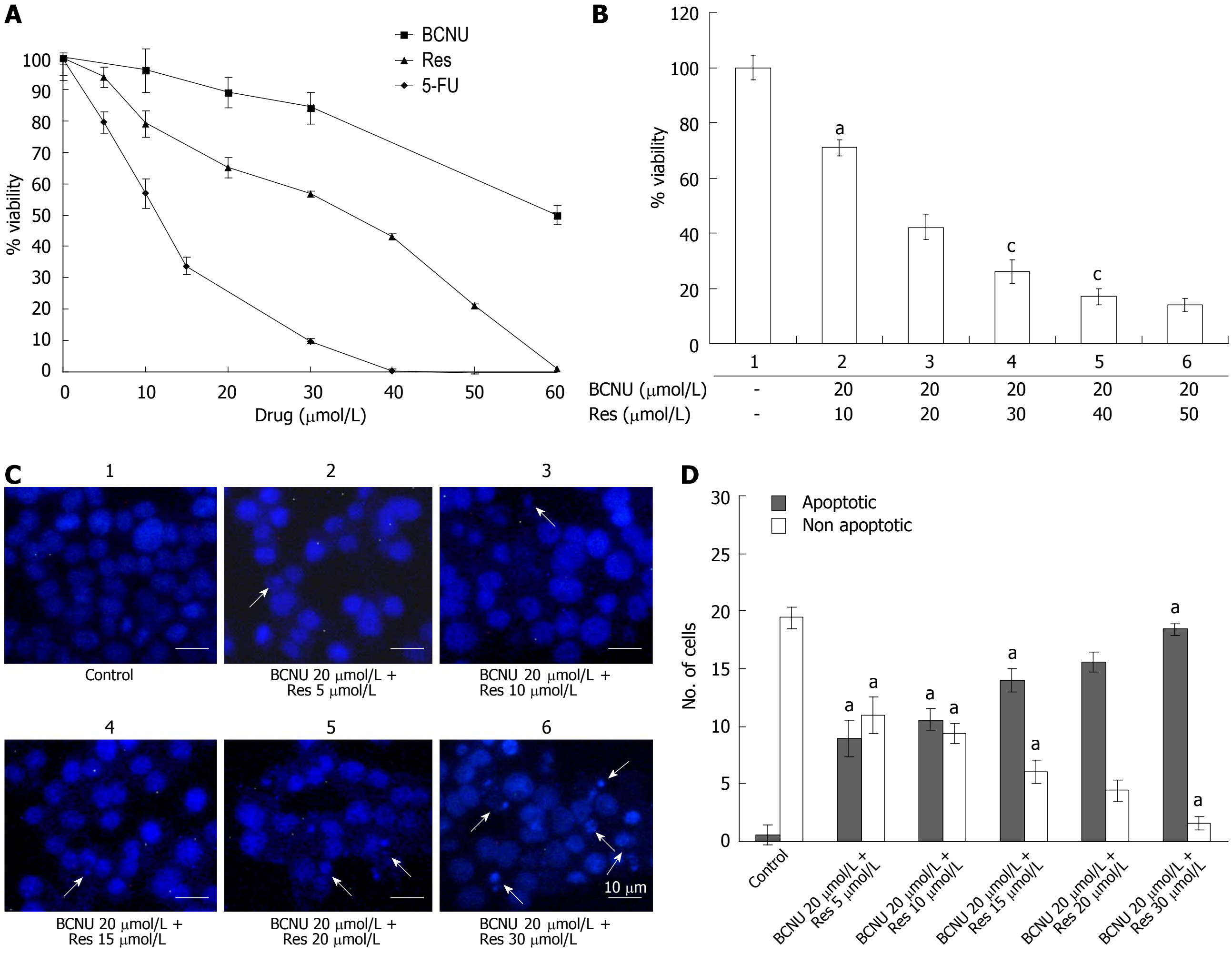


Figure 1 Anti-proliferative and apoptotic effect of 1,3-Bis(2-chloroethyl)-1-nitrosourea and/or resveratrol on HCT-116 colon cancer cells. A: Anchorage-dependent cell survival of HCT-116 cells after treatment with Res, 5-FU and BCNU; B: Bar diagram representing the % viability of HCT-116 after BCNU+ Res exposure. HCT-116 cells were cultured in 96 well plates and grown to 60%-70% confluence. The cells were then treated with different compounds according to the materials and methods. Data are the mean ± SD of three different experiments. $^{a}P < 0.05$ *vs* 20 μmol/L BCNU + 20 μmol/L Res; $^{c}P < 0.05$ *vs* 20 μmol/L BCNU + 50 μmol/L Res; C: Apoptotic nuclei after 4',6'-diamidino-2-phenylindole hydrochloride staining. Images were taken using a fluorescent microscope (Nikon-Eclipse, Japan) at × 40 magnification. Arrows indicate the apoptotic nuclei. Data are the representation of one of the replicates of three different experiments; D: A graphical representation of apoptotic nuclei. $^{a}P < 0.05$ *vs* 20 μmol/L BCNU + 20 μmol/L Res. 5-FU: 5-fluorouracil; Res: Resveratrol; BCNU: 1,3-Bis(2-chloroethyl)-1-nitrosourea.

assays (single cell gel electrophoresis or comet assay). The cells were pre-treated with 20 μmol/L BCNU for 24 h prior to exposure with various concentrations of Res for another 48 h. The comet formation and average comet length increased with increasing concentrations of Res (Figure 2A). Figure 2B shows the average comet length of combined treatment. Compared with untreated cells, the average comet length increased when Res was combined with BCNU (Figure 2B) ($P < 0.05$). Thus, the data suggests that the DNA damaging effect of Res was magnified in presence of BCNU.

Effects of BCNU and Res on the cell cycle regulation and apoptosis of HCT-116

It was reported that Res halts the cell cycle at S phase in various cancer cells, such as breast, colon and pancreas[45-49]. Similarly, it was also reported that BCNU arrests the cell cycle in the G2/M phase transition[50]. To determine the regulation of cell cycle profile by BCNU + Res combination, we treated the cells with the above-mentioned drugs and performed FACS analysis at the end of the exposure. The percentages of G2/M population of cells decreased with increasing concentrations of Res combined with BCNU in comparison with the control (Figure 2C). The percentages of apoptotic cells (sub G_1) population increased in a dose dependent manner with Res combined with a fixed concentration of BCNU. Interestingly, approximately 60% apoptosis was noted when 30 μmol/L Res was combined with 20 μmol/L of BCNU (Figure 2C).

Effects of BCNU and Res on the expression level of apoptotic markers in HCT-116 cells

The combined effect of BCNU and Res on apoptosis, cell cycle regulation and DNA damage repair proteins in HCT-116 cells was studied by measuring the protein expression levels of well known markers, such as Bcl-2-associated X protein (BAX), B-cell lymphoma-extra large

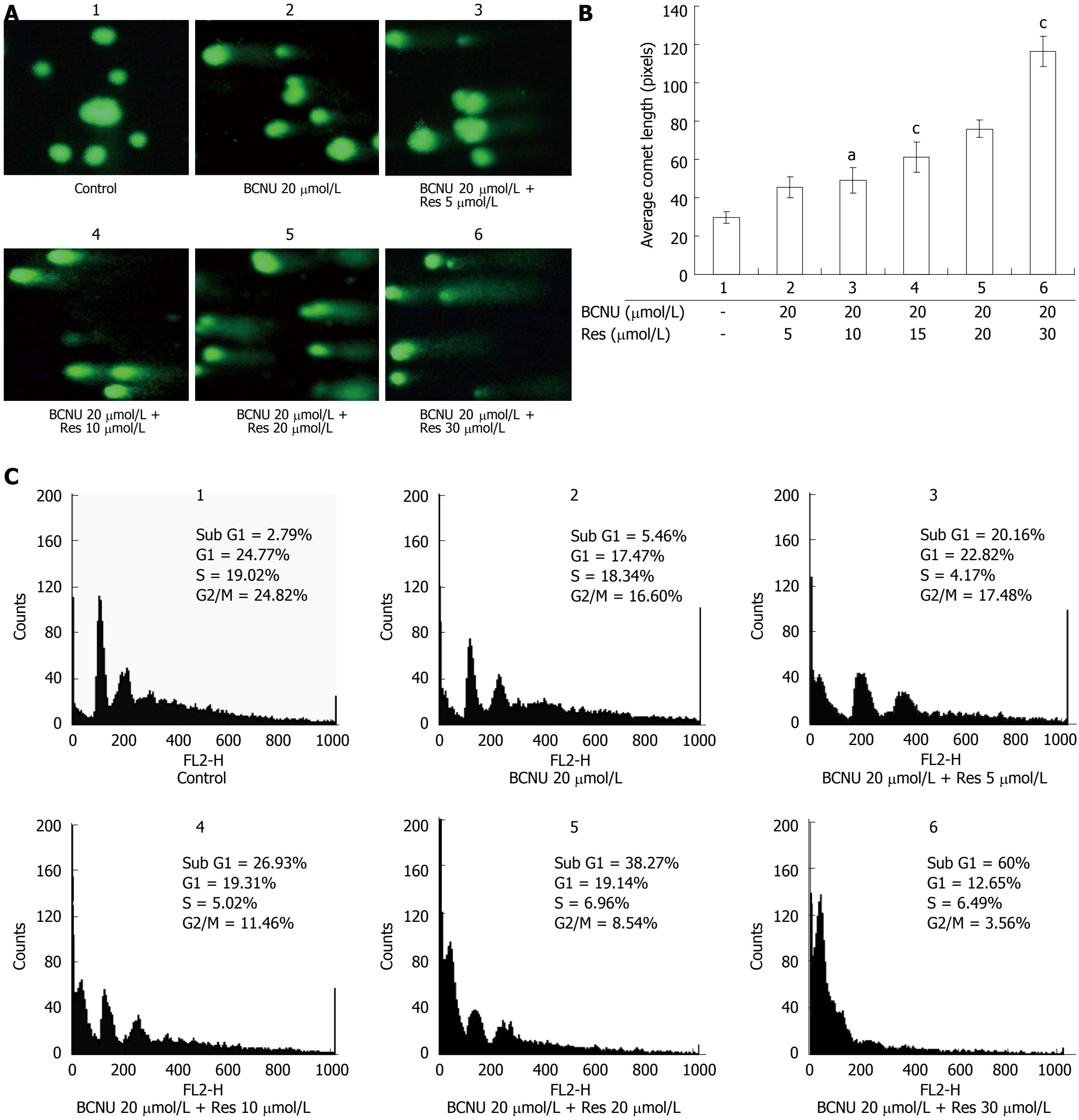


Figure 2 Regulation of cell cycle and genotoxicity of HCT-116 cells after 1,3-Bis(2-chloroethyl)-1-nitrosourea and/or resveratrol treatments. A: Comet assay showing the DNA damaging effect of the drugs in HCT-116 cells. Images were taken using a fluorescent microscope (Nikon-Eclipse, Japan) at × 20 magnification. Data are the representation of one of the replicates of three different experiments; B: Bar diagram represents the average comet - length in pixels as obtained from TriTek CometScore™ software. Data are the mean ± SD of three different experiments, $^{a}P < 0.05$ *vs* 20 μmol/L BCNU + 5 μmol/L Res; $^{c}P < 0.05$ *vs* 20 μmol/L BCNU + 20 μmol/L Res; C: Effect of the drugs on cell cycle regulation. After treatment as mentioned in the materials and method fluorescence-activated cell sorting analysis was performed and the DNA content of the cell was measured by Cell Quest Software (Becton and Dickinson, CA). Data are the representation of one of the replicates of three different experiments. 5-FU: 5-fluorouracil; Res: Resveratrol; BCNU: 1,3-Bis(2-chloroethyl)-1-nitrosourea.

(BCL-XL), Poly (ADP-ribose) polymerase (PARP), p21, proliferating cell nuclear antigen (PCNA), APE, phosphatase and tensin homolog (PTEN), FEN1 and human homolog of cyclin dependent kinase-2 (CDC-2) (Figure 3). The level of BAX increased compared with treatment with BCNU alone, whereas the level of BCL-XL decreased (Figure 3). The levels of CASPASE-3 and cleaved product of PARP also increased compared with BCNU alone (Figure 3). Thus, the data indicated that treatment with the BCNU and Res combination results in apoptosis in HCT-116 cells, as reflected by the elevated BAX/BCL-XL ratio, PARP cleavage and CASPASE-3 expression (Figure 3).

Effects of BCNU and Res on the expression level of cell cycle regulatory and DNA repair proteins in HCT-116 cells

DNA damage repair proteins, such as FEN1, APE, and

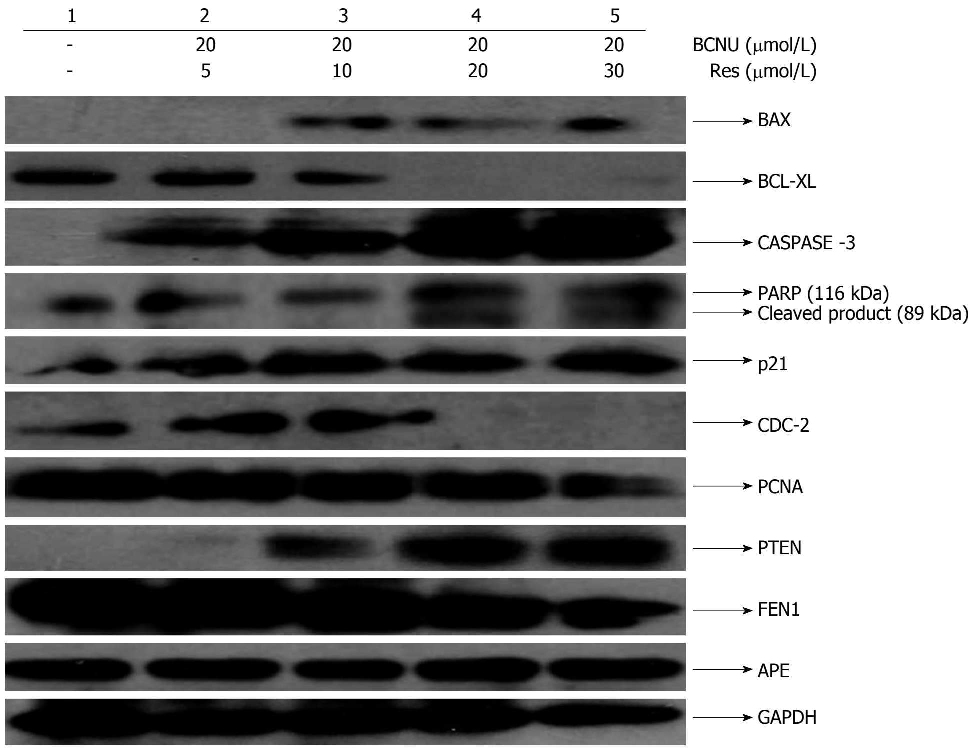


Figure 3 Combined effects of 1,3-Bis(2-chloroethyl)-1-nitrosourea and resveratrol on various cellular markers in HCT-116 colon cancer cells. Expression pattern of apoptotic, DNA damage/repair and cell cycle regulatory proteins after drug treatment. Data are the representation of one of the replicates of three different experiments. Glyceraldehyde phosphate dehydrogenase (GAPDH) served as a loading control. BAX: Bcl-2-associated X protein; BCL-XL: B-cell lymphoma-extra large; PARP: Poly (ADP-ribose) polymerase; PCNA: Proliferating cell nuclear antigen; PTEN: Phosphatase and tensin homolog; FEN1: Flap endonuclease 1; CDC-2: Cyclin dependent kinase-1; Res: Resveratrol; BCNU: 1,3-Bis(2-chloroethyl)-1-nitrosourea; APE: Apurinic/apyrimidinic (AP) endonuclease.

PCNA, were assayed to determine the effects on DNA damage/repair in HCT-116 cells after BCNU and Res combination treatment (Figure 3). FEN1 and PCNA levels decreased when the dose of Res increased, whereas the level of APE remained almost constant during combination treatment (Figure 3). The cell cycle regulatory protein p21 and PTEN increased, while CDC-2 decreased, after combined treatment for 48 h. PTEN is considered to be a cell cycle regulatory tumor suppressor protein, and an increase in PTEN expression indicates the anti-tumor property of the combined drug. Increased p21 expression during the combination treatment indicated negative cell cycle regulation. A decrease in CDC-2 expression also indicated negative regulation of the cell cycle. Thus, these experiments demonstrated that treatment with a combination of BCNU and Res induced apoptosis in colon cancer cells by affecting cell cycle regulation and DNA damage/repair. Therefore, this novel drug combination can be used for treating 5-FU resistant cells.

Establishment of a 5-FU-resistant stable cell line

The 5-FU-R cell line was further characterized by measuring the expression pattern of anti-apoptotic, cell cycle and DNA damage repair proteins, such as nuclear factor kappa-light-chain-enhancer of nuclear factor κB (NFκB), FLICE-like inhibitory protein (FLIP), casein kinase 1 (CK1), disabled homolog 2 (DAB2), glycogen synthase kinase 3 beta (GSK-3β), AKT, POLH, TS, POL-β, DDB2, and FEN1, which were altered in stable cell line compared to normal HCT-116 cells (Figure 4). The expression levels of AKT, NFκB, FLIP, POLH, GSK-3β, POL-β, DDB2, and FEN1 were increased in comparison with HCT-116 cells. However, the levels of CK1 and DAB2 expressions decreased compared with HCT-116 cells (Figure 4). Interestingly, widely established 5-FU resistance protein thymidylate synthase also increased in 5-FU-R cells compared with HCT-116 cells (Figure 4).

Cytotoxicity of the BCNU and Res combination in 5-FU-R cells

The experiments discussed above indicated that BCNU and Res combination significantly increased the sensitivity of HCT-116 colon cancer cells. To determine the optimum concentration of the BCNU and Res combination for cytotoxicity of 5-FU-R cells, an MTT assay was carried out. BCNU showed similar cytotoxic profile in 5-FU-R cells compared with HCT-116 cells (LC_{50} 55 μmol/L) (Figure 5A); however, it was noted that 5-FU resistant cells were more sensitive to Res compared with HCT-116 parental cells. The LC_{50} of Res in 5-FU-R cells was 18 μmol/L, but was 35 μmol/L in HCT-116 cells (Figure1A *vs* Figure 5A). Interestingly, it was noted that there was little or no effect of 5-FU on 5-FU-R cells, even after treatment with 100 μmol/L for 48 h (Figure 5A). This data suggests that 5-FU-R cells are resistant to 5-FU, but sensitive to other chemotherapeutic drug, such

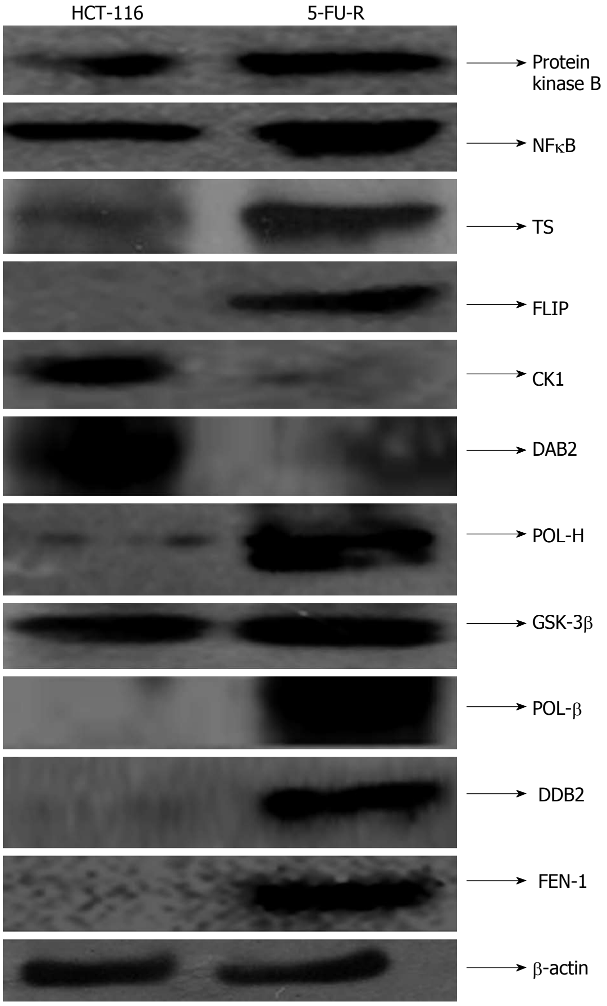


Figure 4 Characterization of 5-fluorouracil-resistant cells. The cell lysates of HCT-116 and 5-FU-R cells were immunoblotted using specific antibody. The lower panel shows the expression of β-actin, which was the loading control (to ensure the same amount of protein loaded in each lane). Data are the representation of one of the replicates of three different experiments. CK1: Casein kinase 1; FLIP: FLICE-like inhibitory protein; NFκB: nuclear factor κB; POL-β: DNA polymerase beta; POLH: DNA polymerase eta; protein Flap FEN1: Endonuclease 1; DDB2: DNA damage-binding protein 2; 5-FU: 5-fluorouracil; Res: Resveratrol; BCNU: 1,3-Bis(2-chloroethyl)-1-nitrosourea; TS: Thymidylate synthase.

as Res.

A dose dependent decrease in cell viability was observed when increasing concentrations of Res were added to the 20 μmol/L BCNU pre-treated 5-FU-R cells (Figure 5B). Fifty percent cell death (LC_{50}) was noticed when 8 μmol/L of Res was combined with 20 μmol/L of BCNU (Figure 5B) ($P < 0.05$). Interestingly, it was noted that lower concentrations of Res were needed to cause fifty percent cell death in 5-FU-R cells compared with HCT-116 cells (LC_{50} for HCT-116 cells was 18 μmol/L *vs* LC_{50} for 5-FU-R cells was 8 μmol/L). Thus, it appeared that 5-FU-R resistant cells (Figure 5B) were more susceptible than HCT-116 cells (Figure 2B) to the BCNU and Res combination.

Apoptosis of 5-FU-R cells was measured by DAPI nuclear staining after exposure to the optimum formulation (BCNU 20 μmol/L + Res 8 μmol/L), BCNU and Res. Although treatment with BCNU and Res alone caused cell death, increased cell death was observed by treatment with the combination compared with BCNU and Res alone (Figure 5C). However, Res alone caused more apoptosis than BCNU alone (Figure 5C). Figure 5D shows the graphical representation of the number of apoptotic and non-apoptotic nuclei ($P < 0.05$).

Combined effect of BCNU and Res caused increased DNA damage, and decreased cell cycle regulatory protein expression and apoptosis in 5-FU-R cells

To elucidate the underlying mechanism of the sensitivity 5-FU-R cells to the BCNU and Res combination, a series of experiments were carried out. To determine whether the BCNU and Res combination caused DNA damage, a comet assay was performed after treatment with BCNU 20 μmol/L + Res 8 μmol/L for 48 h. The average comet length and number of comets increased with the combination treatment compared with BCNU and Res alone or untreated cells (Figure 6A). Figure 6B shows the average comet length, in pixels, of 5-FU-R cells treated with BCNU and Res alone and in combination [BCNU (20 μmol/L) +Res (8 μmol/L)] ($P < 0.05$). The average comet length almost doubled when 8 μmol/L of Res was combined with BCNU compared with BCNU alone. The percentage of apoptosis and cell cycle profile cells were also measured by FACS analysis after treatment with the combined drugs (Figure 6C). Increased accumulation of the G_2/M (24%) population was noted after the BCNU treatment in comparison to Res (19.33%) and untreated cells (10%). Interestingly, the increase in G_2/M population decreased when Res was combined with BCNU; however, the observed percentage of apoptosis (sub G_1) was as high as 70%. To further confirm the apoptosis and to determine the status of various protein biomarkers, Western blotting was carried out. Figure 6D shows the expression patterns of apoptotic markers, such as cleaved product of PARP, BAX, CASPASE-3 and BCL-XL. The cleaved product of PARP and the BAX/BCL-XL ratio increased with increasing concentration of the combined drug. Cell cycle regulatory proteins, such as p21 and PTEN increased, while CDC-2 decreased after combined treatment for 48 h, indicating that this drug combination has adverse effect on cell cycle regulation in the 5-FU-R cells. Figure 6E represents the expression pattern of DNA repair proteins when treated with the combined drug and Res and BCNU alone. The levels of FEN1, POLH, DDB2 and POL-β were decreased in cells that were treated with BCNU and Res alone compared with the control, but the combination treatment showed a radical decrease in the levels of these proteins compare with the control and treatment with each drug alone (Figure 6E). Interestingly, the level of tumor suppressor and DNA repair protein APC was completely abolished after individual (BCNU and Res) treatment, but increased after combined drug exposure compared with untreated cells. This result clearly indicates that the BCNU and Res works additively or synergistically and their mode of ac-

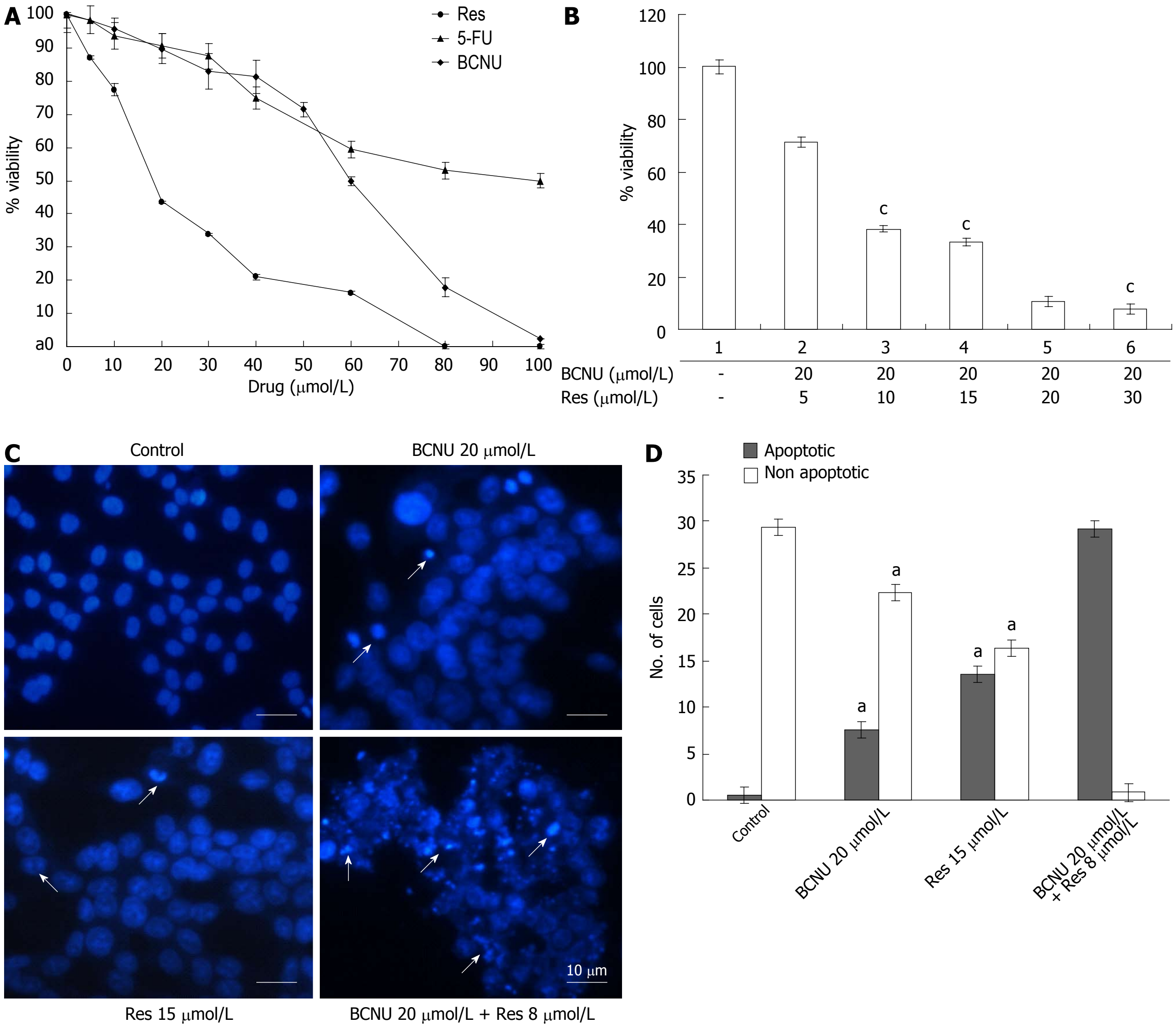


Figure 5 Anti-proliferative and apoptotic effect of 3-Bis(2-chloroethyl)-1-nitrosourea and/or resveratrol on 5-fluorouracil-R cells. A: Anchorage-dependent cell survival of 5-FU-R cells after treatment with 5-FU, Res and BCNU; B: Bar diagram representing the % viability of 5-FU-R cells after BCNU + Res exposure. Data are the mean ± SD of three different experiments, $^{a}P < 0.05$ *vs* 20 μmol/L BCNU + 5 μmol/L Res; $^{c}P < 0.05$ *vs* 20 μmol/L BCNU + 20 μmol/L Res; C: Apoptotic nuclei after DAPI staining. Images were taken using a fluorescent microscope (Nikon-Eclipse, Japan) at ×40 magnification. An arrow indicates the apoptotic nuclei. Data are the representation of one of the replicates of three different experiments; D: A graphical representation of apoptotic nuclei, $^{a}P < 0.05$ *vs* 20 μmol/L BCNU + 8 μmol/L Res. 5-FU: 5-fluorouracil; Res: Resveratrol; BCNU: 1,3-Bis(2-chloroethyl)-1-nitrosourea.

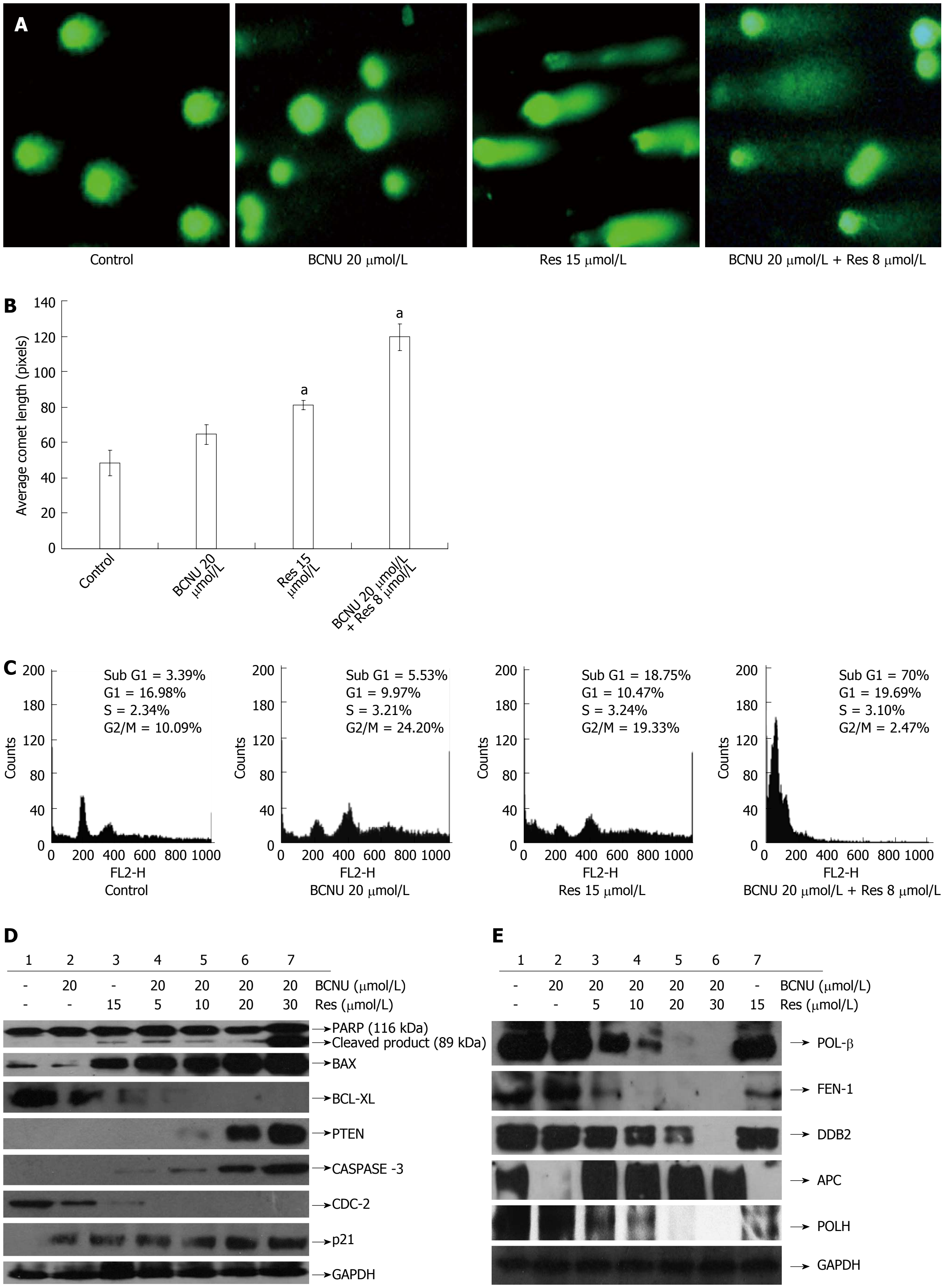
tion was to modulate DNA damage/repair pathway.

BCNU and Res synergistically increase the sensitivity in human colon cancer cells

The above data showed that the BCNU and Res combination increased the sensitivity of colon cancer cells. To determine whether these two drug work additively or synergistically, we performed isobologram analysis. Figure 6F shows the occurrence of a synergistic interaction of Res and BCNU in both the HCT-116 and 5-FU-R cell lines. The LC_{50} value of the Res and BCNU in HCT-116 cells were 30 and 60 μmol/L, respectively, but for the combination, fifty percent cell death occurred at 20 μmol/L BCNU and 18 μmol/L Res (Figure 2B), which were much lower than dose of each individual drug (point Q). Point Q appeared below the lines, which suggested a synergistic interaction of these drugs. To further investigate the synergistic interaction of these drugs against the 5-FU-R cells, the same isobologram was plotted using the LC_{50} value of MTT data from Figure 5B. Interestingly, the fifty percent cell death (point P) with the combined drug appeared below the line, clearly indicating synergistic activity of BCNU and Res in 5-FU-R cells (Figure 6F).

DISCUSSION

5-FU is widely used for the treatment of many types of cancers, including colorectal, breast, and cancers of the aerodigestive tract. Notably, 5-FU is routinely employed in the management of colorectal cancer *via* one of the two FDA-approved first line combinatorial chemothera-


A
Control
BCNU 20 μmol/L
Res 15 μmol/L
BCNU 20 μmol/L + Res 8 μmol/L
B
Average comet length (pixels)
0
20
40
60
80
100
120
140
a
a
Control
BCNU 20 μmol/L
Res 15 μmol/L
BCNU 20 μmol/L + Res 8 μmol/L
C
Sub G1 = 3.39%
G1 = 16.98%
S = 2.34%
G2/M = 10.09%
Sub G1 = 5.53%
G1 = 9.97%
S = 3.21%
G2/M = 24.20%
Sub G1 = 18.75%
G1 = 10.47%
S = 3.24%
G2/M = 19.33%
Sub G1 = 70%
G1 = 19.69%
S = 3.10%
G2/M = 2.47%
Counts
0
40
80
120
180
200
0
200
400
600
800
1000
FL2-H
Control
BCNU 20 μmol/L
Res 15 μmol/L
BCNU 20 μmol/L + Res 8 μmol/L
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1 2 3 4 5 6 7
- 20 - 20 20 20 20 BCNU (μmol/L)
- - 15 5 10 20 30 Res (μmol/L)
PARP (116 kDa)
Cleaved product (89 kDa)
BAX
BCL-XL
PTEN
CASPASE -3
CDC-2
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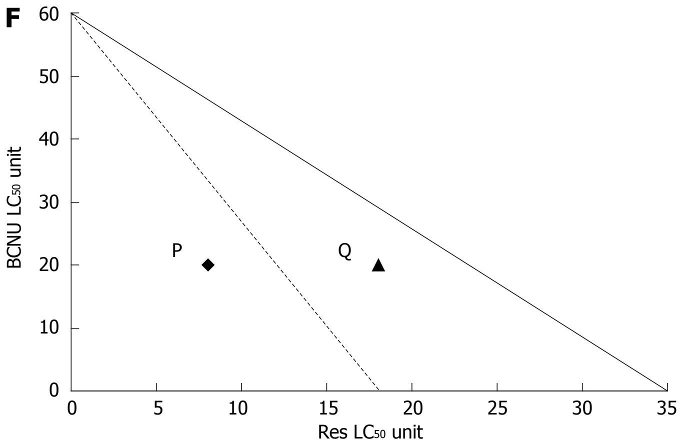

Figure 6 1,3-Bis(2-chloroethyl)-1-nitrosourea and Res synergistically increased apoptosis in 5-fluorouracil-resistance cells. A: Comet assay. Images were taken using a fluorescent microscope (Nikon-Eclipse, Japan) at × 20 magnification. Data are the representation of one of the replicates of three different experiments; B: Bar diagram representing the average comet score of the treated and untreated 5-FU-R cells in pixels, as obtained from TriTek CometScore™ software. Data are the mean ± SD of three different experiments, $^aP < 0.05$ *vs* 20 μmol/L BCNU; C: Regulation of cell cycle and apoptosis by BCNU, Res and their combination in 5-FU-R cells. Fluorescence-activated cell sorting analysis was done after the desired treatment and then the DNA content was measured by Cell Quest Software. Data are the representation of one of the replicates of three different experiments; D, E: Western blotting of 5-FU-R cell lysate after treatment with BCNU and Res individually, as well as in combination. Glyceraldehyde-3-phosphate dehydrogenase was used as the loading control. Data are the representation of one of the replicates of three different experiments; F: Isobolograms for combinations of BCNU and Res in HCT-116 and 5-FU-R cells. The bold line represents the isobologram of BCNU and Res in HCT-116 and dotted lines represents the isobologram of BCNU and Res in 5-FU-R cells. The point P and Q represent the 50% cell death of the combined drug in case of 5-FU-R and HCT-116 cells, respectively. 5-FU: 5-fluorouracil; Res: Resveratrol; BCNU: 1,3-Bis(2-chloroethyl)-1-nitrosourea.

py regimes, which involve intravenous administration of the fluorinated base analog[10,11]. One problem with 5-FU treatment is that more than 50% of tumors demonstrate resistance to5-FU in the clinical setting. Thus, novel treatments are needed to treat these resistant tumors. Novel biological agents, such as the monoclonal antibodies Cetuximab (an epidermal growth factor receptor inhibitor) and Bevacizumab (a vascular endothelial growth factor inhibitor), have recently been shown to provide additional clinical benefit for patients with metastatic colorectal cancer. However, these agents have demonstrated marginal results when treating resistant tumors[51,52].

During the past few decades, investigators have attempted to elucidate the mechanism underlying 5-FU resistance[10-12]. Some investigators developed 5-FU resistant cell lines and studied their sensitivity to multiple chemotherapeutic agents by activation and inhibition of several signaling pathways[53,54]. Unfortunately, none of these agents showed promising results.

In the present report, we developed a new strategy for overcoming 5-FU resistance by establishing 5-FU-R cell lines and studying their sensitivity to a combination treatment with BCNU and Res. BCNU and Res display anticancer properties by inducing DNA damage, arresting cell cycle progression, inhibiting anti-apoptotic makers and autophagy[13,28]. We have shown recently that moderately low concentration of 5-FU (0.5 μmol/L) increased chemoprevention potential of Res in colon cancer cells[7]. However, in that report we could not eliminate the probability of 5-FU resistance because of presence of 5-FU. Thus, that cocktail will not be appropriate for the treatment of 5-FU resistance patients. In the present study, we treated the cells with BCNU and Res to increase the sensitivity of 5-FU resistant cell lines. Initially, we checked whether BCNU and Res combination increase the sensitivity of 5-FU sensitive HCT-116 cells. LC_{50} was observed at 60 μmol/L BCNU and 35 μmol/L Res; however, the same amount of cell death was observed when 18 μmol/L Res was combined with 20 μmol/L BCNU. Reduction of both BCNU and Res concentration for fifty percent cell death revealed that BCNU and Res killed HCT-116 colon cancer cells synergistically (Figure 1B). The isobologram data (Figure 6F) also support the synergistic action of BCNU and Res to kill colon cancer cells.

Cell death associated with the synergistic effects of treating cells with a combination of BCNU and Res was further confirmed by multiple parameters, such as DAPI nuclear staining, comet assay, FACS analysis and measuring the expression patterns of several DNA damage/repair, cell cycle regulatory and apoptosis related protein biomarkers. Increased numbers of apoptotic nuclei after DAPI stain, average comet formation and comet length, percentage of sub G_1 population were observed after combined treatment (BCNU + Res) in HCT-116 cells. An increased ratio of BAX/BCL-XL, cleaved product of PARP, tumor suppressor PTEN, cell cycle regulatory protein p21, and CASPASE-3 were also noted in BCNU and Res combination. Thus, the data suggests that the BCNU and Res combination represents a potential treatment option for 5-FU resistant colon cancer.

We have developed a 5-FU resistant cell line from 5-FU sensitive HCT-116 cells by continuous treatment with 5-FU. These cells were resistant to 5-FU, but sensitive to other chemotherapeutic agents, such as Res. The cell death effects of the BCNU and Res combination in the resistant cell lines were measured. MTT cell survival, DAPI nuclear staining and comet assays showed that the

BCNU + Res combination was highly effective to sensitize the 5-FU-R cells in comparison to individual compounds. They caused cell death synergistically. The FACS analysis showed approximately 70% cell death when 20 μmol/L BCNU combined with 8 μmol/L Res (Figure 6C). By contrast, approximately 60% cell death was observed when 20 μmol/L of BCNU combined with 30 μmol/L Res in HCT-116 sensitive cells. Increased percentages (70% *vs* 60%) of apoptosis even at lower concentration of Res (8 μmol/L *vs* 30 μmol/L) indicated that Res was more effective to sensitize the 5-FU-R than HCT-116 cells when combined with BCNU. The expression of PARP cleaved product and BAX/BCL-XL ratio increased with the combined treatment (BCNU + Res) in 5-FU-R cells compared with Res and BCNU alone (Figure 6D). Isobologram analysis also indicated that BCNU and Res also killed the 5-FU-R cells synergistically.

To study the mechanism of action of the BCNU and Res combination, we have first analyzed whether the DNA damage/repair pathway was involved. Comet assays showed that the average comet length and comet formation increased in both 5-FU sensitive and resistant cell lines after treatment with the BCNU + Res combination. Interestingly, a much lower concentration of Res (8 μmol/L) was needed to cause the same comet length in 5-FU-R cells compared with 5-FU sensitive cells (Res 30 μmol/L) (Figure 2B *vs* Figure 6B). This observation suggests that Res was more effective in causing DNA damage in 5-FU-R cells than in 5-FU sensitive cells when associated with the same amount of BCNU. This data prompted us to check the level of DNA damage/repair related proteins after treatment with BCNU + Res in 5-FU-R cells. Interestingly, the major DNA repair proteins, such as FEN1, POLH, DDB2 and POL-β decreased in case of combination treatment while the level of APC increased in comparison to the individual drug as well as untreated cells (Figure 6E).

Multiple pieces of evidence indicate that the repair of apurinic/apyrimidinic (AP)-sites in DNA occurs through two sub-pathways of BER, which differ on the basis of repair gap size and the enzymes involved in these repair pathways[55]. These sub-pathways are designated as “single-nucleotide BER” or ”short patch (SP)-BER” and “multinucleotide BER” or ”long patch (LP)-BER”. In both pathways, repair is started by removal of a damaged base by a DNA glycosylase leaving an abasic (AP-site) DNA. The resulting AP sites are subsequently acted upon by an APE to generate a 3’ hydroxyl group and a 5’-deoxyribosephosphate (dRP) terminus. In SP-BER, Pol-β extends the 3’ terminus by a single nucleotide and removes the dRP moiety with its dRP lyase activity. Finally, the nick is sealed by DNA ligases[56]. However, the oxidized or reduced AP sites become resistant to β-elimination and cannot be excised by the dRP lyase activity of Pol-β. Then the modified AP-site is repaired *via* the LP-BER pathway, in which Pol-β, d or ε incorporates 2-15 nucleotides, displacing the strand containing the modified AP-site. Then, the DNA flap structure is cleaved by FEN1, and the nick is sealed by a DNA ligase[57,58]. APC expression is induced in cancer cell lines upon exposure to DNA-damaging agents[39,40,59,60], suggesting an interaction between APC and the DNA repair machinery. We have already shown that APC physically interacts with Pol-β, FEN1 and blocks BER by blocking strand-displacement synthesis and then DNA repair[39-42]. Increased levels of APC and decreased levels of Pol-β and FEN1 after combined treatment with BCNU + Res revealed that this cocktail kills cancer cells by inhibiting the BER pathway. Thus, the above result indicates that BCNU + Res in combination caused cell death through reduction of BER repair pathway. The possible cause of this effect might be activation of APC by the combination but not the individual treatments.

A new and emerging concept is to sensitize cancer cells to DNA-damaging agents by inhibiting various proteins in DNA repair pathways. Molecular docking or NMR studies identified small molecular weight inhibitors (SMIs) that target the BER pathway by inhibiting APE1 and Pol-β activities[61]. Although a number of Pol-β inhibitors have been reported in recent years[61,62], more potent and selective inhibitors are still needed. Since abasic DNA damage (which may also be caused by BCNU) can also be repaired by LP-BER, there is a need for agents that can block the LP-BER pathway as well, in which 5’-flap endonuclease 1 (FEN1) plays a major role[63]. FEN1 recognizes and removes the 5’-flap structure generated by Pol-β during the strand-displacement synthesis. The removal of this flap is essential for the joining of the newly synthesized DNA strand with the parent strand by DNA ligase to complete the repair. Interestingly, the FEN1 level decreased after BCNU and Res combination in both cell lines, indicating that the BCNU + Res combination inhibited the removal of flap from the newly synthesized step in LP-BER. BCNU + Res might cause more DNA damage that could not be repaired by the cells because of a lack of sufficient FEN1. This would result in mutations accumulating within the cells, eventually leading to apoptosis instead of survival. However, more study will be needed to understand the molecular mechanism involving the BER pathway triggered by the BCNU + Res combination.

In conclusion, this study showed that a combination of BCNU and Res could be useful to overcome 5-FU resistance in colon cancer patients. BCNU and Res caused apoptosis in both the HCT-116 cells and 5-FU-R cells by inhibiting the base excision repair, especially *via* the FEN1 protein of the LP-BER pathway. These findings represent a novel concept for overcoming 5-FU resistance in colon cancer patients.

COMMENTS

Background

Colon cancer is one of the most common causes of cancer-related death worldwide. 5-fluorouracil (5-FU) is a widely used chemotherapeutic drug for the treatment of colon cancer, but resistance to this drug is a barrier to successful chemotherapy.

Research frontiers

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) is a synthetic drug commonly used to treat brain tumors as it can pass through the blood-brain barrier. BCNU is highly effective against cancer, but causes toxicity at very high concentrations. Resveratrol is a plant-derived compound used to treat various forms of cancer. Res has low toxicity and efficacy. Combining BCNU with resveratrol (Res) increases the efficacy of Res and reduces the toxicity of BCNU, making the combination an good tool to inhibit HCT-116 cells and overcome 5-FU resistance. Moreover, these drugs are DNA damage specific, resembling similar mechanism of action to that of 5-FU by reducing DNA repair components. Hence, these drugs were chosen for the combination therapy.

Innovations and breakthroughs

This report highlighted the importance of combination therapy, which surpassed the monotherapy involving BCNU and Res to inhibit the growth of HCT-116 cells and overcome 5-FU resistance. This is the first study to report a combination of BCNU and Res for the treatment of colon cancer and to overcome 5-FU resistance effectively. The authors reported the mechanism of action of the combined drug, *i.e.*, inhibition of the DNA damage/repair pathway and arresting the cell cycle, thereby leading to apoptosis. The authors also reported that the drug to be more effective in the resistant cells than the normal cancer cells.

Applications

This study highlights the action of BCNU combined with Res, which could prove to be a major tool for the treatment of 5-FU sensitive/resistant colon cancer patients and could also be tested for its ability to overcome resistance to other drugs, as this cocktail had specificity towards drug resistant cells.

Terminology

BCNU is a common synthetic alkylating chemotherapeutic agent belonging to the family of haloethylnitrosoureas. Resveratrol is a polyphenolic phytoalexin widely available in plants, such as red grapes.

Peer review

The research work in this manuscript is about the tumor suppression action of BCNU in combination with resveratrol on 5-fluorouracil HCT-116 cell lines, which were made resistant to 5-FU on constant exposure of 5-FU. The study is interesting and well executed. The point is clearly made.

REFERENCES

1 **Boring CC**, Squires TS, Tong T. Cancer statistics, 1991. *CA Cancer J Clin* 1991; **41**: 19-36 [PMID: 1984806 DOI: 10.3322/canjclin.41.1.19]

2 **Glimelius B**, Hoffman K, Graf W, Påhlman L, Sjödén PO. Quality of life during chemotherapy in patients with symptomatic advanced colorectal cancer. The Nordic Gastrointestinal Tumor Adjuvant Therapy Group. *Cancer* 1994; **73**: 556-562 [PMID: 8299077]

3 **Mullins CD**, Hsiao FY, Onukwugha E, Pandya NB, Hanna N. Comparative and cost-effectiveness of oxaliplatin-based or irinotecan-based regimens compared with 5-fluorouracil/leucovorin alone among US elderly stage IV colon cancer patients. *Cancer* 2012; **118**: 3173-3181 [PMID: 22020739 DOI: 10.1002/cncr.26613]

4 **Wang W**, Gallant JN, Katz SI, Dolloff NG, Smith CD, Abdulghani J, Allen JE, Dicker DT, Hong B, Navaraj A, El-Deiry WS. Quinacrine sensitizes hepatocellular carcinoma cells to TRAIL and chemotherapeutic agents. *Cancer Biol Ther* 2011; **12**: 229-238 [PMID: 21725212 DOI: 10.4161/cbt.12.3.17033]

5 **Patel BB**, Sengupta R, Qazi S, Vachhani H, Yu Y, Rishi AK, Majumdar AP. Curcumin enhances the effects of 5-fluorouracil and oxaliplatin in mediating growth inhibition of colon cancer cells by modulating EGFR and IGF-1R. *Int J Cancer* 2008; **122**: 267-273 [PMID: 17918158 DOI: 10.1002/ijc.23097]

6 **de Gramont A**, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000; **18**: 2938-2947 [PMID: 10944126]

7 **Mohapatra P**, Preet R, Choudhuri M, Choudhuri T, Kundu CN. 5-fluorouracil increases the chemopreventive potentials of resveratrol through DNA damage and MAPK signaling pathway in human colorectal cancer cells. *Oncol Res* 2011; **19**: 311-321 [PMID: 21936401 DOI: 10.3727/096504011X13079697132844]

8 **Rougier P**, Ducreux M, Mahjoubi M, Pignon JP, Bellefqih S, Oliveira J, Bognel C, Lasser P, Ychou M, Elias D. Efficacy of combined 5-fluorouracil and cisplatinum in advanced gastric carcinomas. A phase II trial with prognostic factor analysis. *Eur J Cancer* 1994; **30A**: 1263-1269 [PMID: 7999410 DOI: 10.1016/0959-8049(94)90170-8]

9 **Longley DB**, Latif T, Boyer J, Allen WL, Maxwell PJ, Johnston PG. The interaction of thymidylate synthase expression with p53-regulated signaling pathways in tumor cells. *Semin Oncol* 2003; **30**: 3-9 [PMID: 12802789 DOI: 10.1016/S0093-7754(03)00119-2]

10 **Wyatt MD**, Wilson DM. Participation of DNA repair in the response to 5-fluorouracil. *Cell Mol Life Sci* 2009; **66**: 788-799 [PMID: 18979208 DOI: 10.1007/s00018-008-8557-5]

11 **Luo Y**, Walla M, Wyatt MD. Uracil incorporation into genomic DNA does not predict toxicity caused by chemotherapeutic inhibition of thymidylate synthase. *DNA Repair (Amst)* 2008; **7**: 162-169 [PMID: 17942376 DOI: 10.1016/j.dnarep.2007.09.001]

12 **Li L**, Connor EE, Berger SH, Wyatt MD. Determination of apoptosis, uracil incorporation, DNA strand breaks, and sister chromatid exchanges under conditions of thymidylate deprivation in a model of BER deficiency. *Biochem Pharmacol* 2005; **70**: 1458-1468 [PMID: 16191427 DOI: 10.1016/j.bcp.2005.08.016]

13 **Filippi-Chiela EC**, Villodre ES, Zamin LL, Lenz G. Autophagy interplay with apoptosis and cell cycle regulation in the growth inhibiting effect of resveratrol in glioma cells. *PLoS One* 2011; **6**: e20849 [PMID: 21695150 DOI: 10.1371/journal.pone.0020849]

14 **Scifo C**, Cardile V, Russo A, Consoli R, Vancheri C, Capasso F, Vanella A, Renis M. Resveratrol and propolis as necrosis or apoptosis inducers in human prostate carcinoma cells. *Oncol Res* 2004; **14**: 415-426 [PMID: 15490973]

15 **Bhardwaj A**, Sethi G, Vadhan-Raj S, Bueso-Ramos C, Takada Y, Gaur U, Nair AS, Shishodia S, Aggarwal BB. Resveratrol inhibits proliferation, induces apoptosis, and overcomes chemoresistance through down-regulation of STAT3 and nuclear factor-kappaB-regulated antiapoptotic and cell survival gene products in human multiple myeloma cells. *Blood* 2007; **109**: 2293-2302 [PMID: 17164350 DOI: 10.1182/blood-2006-02-003988]

16 **Opipari AW**, Tan L, Boitano AE, Sorenson DR, Aurora A, Liu JR. Resveratrol-induced autophagocytosis in ovarian cancer cells. *Cancer Res* 2004; **64**: 696-703 [PMID: 14744787 DOI: 10.1158/0008-5472.CAN-03-2404]

17 **Klionsky DJ**, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, Baba M, Baehrecke EH, Bahr BA, Ballabio A, Bamber BA, Bassham DC, Bergamini E, Bi X, Biard-Piechaczyk M, Blum JS, Bredesen DE, Brodsky JL, Brumell JH, Brunk UT, Bursch W, Camougrand N, Cebollero E, Cecconi F, Chen Y, Chin LS, Choi A, Chu CT, Chung J, Clarke PG, Clark RS, Clarke SG, Clavé C, Cleveland JL, Codogno P, Colombo MI, Coto-Montes A, Cregg JM, Cuervo AM, Debnath J, Demarchi F, Dennis PB, Dennis PA, Deretic V, Devenish RJ, Di Sano F, Dice JF, Difiglia M, Dinesh-Kumar S, Distelhorst CW, Djavaheri-Mergny M, Dorsey FC, Dröge W, Dron M, Dunn WA Jr, Duszenko M, Eissa NT, Elazar Z, Esclatine A, Eskelinen EL, Fésüs L, Finley KD, Fuentes JM, Fueyo J, Fujisaki K, Galliot B, Gao FB, Gewirtz DA, Gibson SB, Gohla A, Goldberg AL, Gonzalez R, González-Estévez C, Gorski S, Gottlieb RA, Häussinger D, He YW, Heidenreich K, Hill JA, Høyer-Hansen M, Hu X, Huang WP, Iwasaki A, Jäättelä M, Jackson WT, Jiang X, Jin S, Johansen T, Jung JU, Kadowaki

M, Kang C, Kelekar A, Kessel DH, Kiel JA, Kim HP, Kimchi A, Kinsella TJ, Kiselyov K, Kitamoto K, Knecht E, Komatsu M, Kominami E, Kondo S, Kovács AL, Kroemer G, Kuan CY, Kumar R, Kundu M, Landry J, Laporte M, Le W, Lei HY, Lenardo MJ, Levine B, Lieberman A, Lim KL, Lin FC, Liou W, Liu LF, Lopez-Berestein G, López-Otín C, Lu B, Macleod KF, Malorni W, Martinet W, Matsuoka K, Mautner J, Meijer AJ, Meléndez A, Michels P, Miotto G, Mistiaen WP, Mizushima N, Mograbi B, Monastyrska I, Moore MN, Moreira PI, Moriyasu Y, Motyl T, Münz C, Murphy LO, Naqvi NI, Neufeld TP, Nishino I, Nixon RA, Noda T, Nürnberg B, Ogawa M, Oleinick NL, Olsen LJ, Ozpolat B, Paglin S, Palmer GE, Papassideri I, Parkes M, Perlmutter DH, Perry G, Piacentini M, Pinkas-Kramarski R, Prescott M, Proikas-Cezanne T, Raben N, Rami A, Reggiori F, Rohrer B, Rubinsztein DC, Ryan KM, Sadoshima J, Sakagami H, Sakai Y, Sandri M, Sasakawa C, Sass M, Schneider C, Seglen PO, Seleverstov O, Settleman J, Shacka JJ, Shapiro IM, Sibirny A, Silva-Zacarin EC, Simon HU, Simone C, Simonsen A, Smith MA, Spanel-Borowski K, Srinivas V, Steeves M, Stenmark H, Stromhaug PE, Subauste CS, Sugimoto S, Sulzer D, Suzuki T, Swanson MS, Tabas I, Takeshita F, Talbot NJ, Tallóczy Z, Tanaka K, Tanaka K, Tanida I, Taylor GS, Taylor JP, Terman A, Tettamanti G, Thompson CB, Thumm M, Tolkovsky AM, Tooze SA, Truant R, Tumanovska LV, Uchiyama Y, Ueno T, Uzcátegui NL, van der Klei I, Vaquero EC, Vellai T, Vogel MW, Wang HG, Webster P, Wiley JW, Xi Z, Xiao G, Yahalom J, Yang JM, Yap G, Yin XM, Yoshimori T, Yu L, Yue Z, Yuzaki M, Zabirnyk O, Zheng X, Zhu X, Deter RL. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy* 2008; **4**: 151-175

18 **Zamin LL**, Filippi-Chiela EC, Dillenburg-Pilla P, Horn F, Salbego C, Lenz G. Resveratrol and quercetin cooperate to induce senescence-like growth arrest in C6 rat glioma cells. *Cancer Sci* 2009; **100**: 1655-1662 [PMID: 19496785 DOI: 10.1111/j.1349-7006.2009.01215.x]

19 **Jiang H**, Zhang L, Kuo J, Kuo K, Gautam SC, Groc L, Rodriguez AI, Koubi D, Hunter TJ, Corcoran GB, Seidman MD, Levine RA. Resveratrol-induced apoptotic death in human U251 glioma cells. *Mol Cancer Ther* 2005; **4**: 554-561 [PMID: 15827328 DOI: 10.1158/1535-7163.MCT-04-0056]

20 **Li J**, Qin Z, Liang Z. The prosurvival role of autophagy in Resveratrol-induced cytotoxicity in human U251 glioma cells. *BMC Cancer* 2009; **9**: 215 [PMID: 19566920 DOI: 10.1186/1471-2407-9-215]

21 **Chen A**. PARP inhibitors: its role in treatment of cancer. *Chin J Cancer* 2011; **30**: 463-471 [PMID: 21718592 DOI: 10.5732/cjc.011.10111]

22 **Panigrahi M**, Das PK, Parikh PM. Brain tumor and Gliadel wafer treatment. *Indian J Cancer* 2011; **48**: 11-17 [PMID: 21330749 DOI: 10.4103/0019-509X.76623]

23 **Ludlum DB**. DNA alkylation by the haloethylnitrosoureas: nature of modifications produced and their enzymatic repair or removal. *Mutat Res* 1990; **233**: 117-126 [PMID: 2233793 DOI: 10.1016/0027-5107(90)90156-X]

24 **Ludlum DB**. The chloroethylnitrosoureas: sensitivity and resistance to cancer chemotherapy at the molecular level. *Cancer Invest* 1997; **15**: 588-598 [PMID: 9412665 DOI: 10.3109/07357909709047601]

25 **Ludlum DB**. Formation of cyclic adducts in nucleic acids by the haloethylnitrosoureas. *IARC Sci Publ* 1986; (**70**): 137-146 [PMID: 3793169]

26 **Kohn KW**. Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. *Cancer Res* 1977; **37**: 1450-1454 [PMID: 851960]

27 **Hang B**, Chenna A, Guliaev AB, Singer B. Miscoding properties of 1,N6-ethanoadenine, a DNA adduct derived from reaction with the antitumor agent 1,3-bis(2-chloroethyl)-1-nitrosourea. *Mutat Res* 2003; **531**: 191-203 [PMID: 14637255 DOI: 10.1016/j.mrfmmm.2003.07.006]

28 **Kuo CC**, Liu TW, Chen LT, Shiah HS, Wu CM, Cheng YT, Pan WY, Liu JF, Chen KL, Yang YN, Chen SN, Chang JY. Combination of arsenic trioxide and BCNU synergistically triggers redox-mediated autophagic cell death in human solid tumors. *Free Radic Biol Med* 2011; **51**: 2195-2209 [PMID: 22001324 DOI: 10.1016/j.freeradbiomed.2011.09.023]

29 **Gajewski TF**, Sosman J, Gerson SL, Liu L, Dolan E, Lin S, Vokes EE. Phase II trial of the O6-alkylguanine DNA alkyltransferase inhibitor O6-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea in advanced melanoma. *Clin Cancer Res* 2005; **11**: 7861-7865 [PMID: 16278409 DOI: 10.1158/1078-0432.CCR-05-0060]

30 **Recinos VR**, Tyler BM, Bekelis K, Sunshine SB, Vellimana A, Li KW, Brem H. Combination of intracranial temozolomide with intracranial carmustine improves survival when compared with either treatment alone in a rodent glioma model. *Neurosurgery* 2010; **66**: 530-57; discussion 537 [PMID: 20173548 DOI: 10.1227/01.NEU.0000365263.14725.39]

31 **Kastan MB**, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 2004; **432**: 316-323 [PMID: 15549093 DOI: 10.1038/nature03097]

32 **Zabludoff SD**, Deng C, Grondine MR, Sheehy AM, Ashwell S, Caleb BL, Green S, Haye HR, Horn CL, Janetka JW, Liu D, Mouchet E, Ready S, Rosenthal JL, Queva C, Schwartz GK, Taylor KJ, Tse AN, Walker GE, White AM. AZD7762, a novel checkpoint kinase inhibitor, drives checkpoint abrogation and potentiates DNA-targeted therapies. *Mol Cancer Ther* 2008; **7**: 2955-2966 [PMID: 18790776 DOI: 10.1158/1535-7163.MCT-08-0492]

33 **Ashwell S**, Zabludoff S. DNA damage detection and repair pathways--recent advances with inhibitors of checkpoint kinases in cancer therapy. *Clin Cancer Res* 2008; **14**: 4032-4037 [PMID: 18593978 DOI: 10.1158/1078-0432.CCR-07-5138]

34 **Wilsker D**, Bunz F. Loss of ataxia telangiectasia mutated- and Rad3-related function potentiates the effects of chemotherapeutic drugs on cancer cell survival. *Mol Cancer Ther* 2007; **6**: 1406-1413 [PMID: 17431119 DOI: 10.1158/1535-7163.MCT-06-0679]

35 **Tse AN**, Carvajal R, Schwartz GK. Targeting checkpoint kinase 1 in cancer therapeutics. *Clin Cancer Res* 2007; **13**: 1955-1960 [PMID: 17404075 DOI: 10.1158/1078-0432.CCR-06-2793]

36 **Xiao Z**, Xue J, Sowin TJ, Zhang H. Differential roles of checkpoint kinase 1, checkpoint kinase 2, and mitogen-activated protein kinase-activated protein kinase 2 in mediating DNA damage-induced cell cycle arrest: implications for cancer therapy. *Mol Cancer Ther* 2006; **5**: 1935-1943 [PMID: 16928813 DOI: 10.1158/1535-7163.MCT-06-0077]

37 **de Angelis PM**, Fjell B, Kravik KL, Haug T, Tunheim SH, Reichelt W, Beigi M, Clausen OP, Galteland E, Stokke T. Molecular characterizations of derivatives of HCT116 colorectal cancer cells that are resistant to the chemotherapeutic agent 5-fluorouracil. *Int J Oncol* 2004; **24**: 1279-1288 [PMID: 15067352]

38 **Narayan S**, Jaiswal AS. Activation of adenomatous polyposis coli (APC) gene expression by the DNA-alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine requires p53. *J Biol Chem* 1997; **272**: 30619-30622 [PMID: 9388195 DOI: 10.1074/jbc.272.49.30619]

39 **Jaiswal AS**, Balusu R, Armas ML, Kundu CN, Narayan S. Mechanism of adenomatous polyposis coli (APC)-mediated blockage of long-patch base excision repair. *Biochemistry* 2006; **45**: 15903-15914 [PMID: 17176113 DOI: 10.1021/bi0607958]

40 **Balusu R**, Jaiswal AS, Armas ML, Kundu CN, Bloom LB, Narayan S. Structure/function analysis of the interaction of adenomatous polyposis coli with DNA polymerase beta and its implications for base excision repair. *Biochemistry* 2007; **46**: 13961-13974 [PMID: 17999539 DOI: 10.1021/bi701632e]

41 **Kundu CN**, Balusu R, Jaiswal AS, Gairola CG, Narayan S.

Cigarette smoke condensate-induced level of adenomatous polyposis coli blocks long-patch base excision repair in breast epithelial cells. *Oncogene* 2007; **26**: 1428-1438 [PMID: 16924228 DOI: 10.1038/sj.onc.1209925]

42 **Preet R**, Mohapatra P, Mohanty S, Sahu SK, Choudhuri T, Wyatt MD, Kundu CN. Quinacrine has anticancer activity in breast cancer cells through inhibition of topoisomerase activity. *Int J Cancer* 2012; **130**: 1660-1670 [PMID: 21544805 DOI: 10.1002/ijc.26158]

43 **Tice RR**, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen* 2000; **35**: 206-221 [PMID: 10737956 DOI: 10.1002/(SICI)1098-2280(2000)35:]

44 **Longley DB**, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; **3**: 330-338 [PMID: 12724731 DOI: 10.1038/nrc1074]

45 **Jang M**, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997; **275**: 218-220 [PMID: 8985016 DOI: 10.1126/science.275.5297.218]

46 **Aziz MH**, Kumar R, Ahmad N. Cancer chemoprevention by resveratrol: in vitro and in vivo studies and the underlying mechanisms (review). *Int J Oncol* 2003; **23**: 17-28 [PMID: 12792772]

47 **Shi Y**, Yang S, Troup S, Lu X, Callaghan S, Park DS, Xing Y, Yang X. Resveratrol induces apoptosis in breast cancer cells by E2F1-mediated up-regulation of ASPP1. *Oncol Rep* 2011; **25**: 1713-1719 [PMID: 21479363 DOI: 10.3892/or.2011.1248]

48 **Miki H**, Uehara N, Kimura A, Sasaki T, Yuri T, Yoshizawa K, Tsubura A. Resveratrol induces apoptosis via ROS-triggered autophagy in human colon cancer cells. *Int J Oncol* 2012; **40**: 1020-1028 [PMID: 22218562 DOI: 10.3892/ijo.2012.1325]

49 **Zhou JH**, Cheng HY, Yu ZQ, He DW, Pan Z, Yang DT. Resveratrol induces apoptosis in pancreatic cancer cells. *Chin Med J (Engl)* 2011; **124**: 1695-1699 [PMID: 21740780 DOI: 10.3760/cma.j.issn.0366-6999.2011.11.017]

50 **Yan L**, Donze JR, Liu L. Inactivated MGMT by O6-benzylguanine is associated with prolonged G2/M arrest in cancer cells treated with BCNU. *Oncogene* 2005; **24**: 2175-2183 [PMID: 15735757 DOI: 10.1038/sj.onc.1208250]

51 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313 DOI: 10.1056/NEJMoa033025]

52 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435 DOI: 10.1056/NEJMoa032691]

53 **Wang W**, Collie-Duguid E, Cassidy J. Cerivastatin enhances the cytotoxicity of 5-fluorouracil on chemosensitive and resistant colorectal cancer cell lines. *FEBS Lett* 2002; **531**: 415-420 [PMID: 12435585 DOI: 10.1016/S0014-5793(02)03575-5]

54 **Zhu H**, Guo W, Zhang L, Davis JJ, Teraishi F, Wu S, Cao X, Daniel J, Smythe WR, Fang B. Bcl-XL small interfering RNA suppresses the proliferation of 5-fluorouracil-resistant human colon cancer cells. *Mol Cancer Ther* 2005; **4**: 451-456 [PMID: 15767554 DOI: 10.1158/1535-7163.MCT-04-0162]

55 **Wilson SH**. Mammalian base excision repair and DNA polymerase beta. *Mutat Res* 1998; **407**: 203-215 [PMID: 9653447 DOI: 10.1016/S0921-8777(98)00002-0]

56 **Srivastava DK**, Berg BJ, Prasad R, Molina JT, Beard WA, Tomkinson AE, Wilson SH. Mammalian abasic site base excision repair. Identification of the reaction sequence and rate-determining steps. *J Biol Chem* 1998; **273**: 21203-21209 [PMID: 9694877 DOI: 10.1074/jbc.273.33.21203]

57 **Bambara RA**, Murante RS, Henricksen LA. Enzymes and reactions at the eukaryotic DNA replication fork. *J Biol Chem* 1997; **272**: 4647-4650 [PMID: 9081985 DOI: 10.1074/jbc.272.8.4647]

58 **Klungland A**, Lindahl T. Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J* 1997; **16**: 3341-3348 [PMID: 9214649 DOI: 10.1093/emboj/16.11.3341]

59 **Narayan S**, Jaiswal AS, Balusu R. Tumor suppressor APC blocks DNA polymerase beta-dependent strand displacement synthesis during long patch but not short patch base excision repair and increases sensitivity to methylmethane sulfonate. *J Biol Chem* 2005; **280**: 6942-6949 [PMID: 15548520 DOI: 10.1074/jbc.M409200200]

60 **Jaiswal AS**, Narayan S. p53-dependent transcriptional regulation of the APC promoter in colon cancer cells treated with DNA alkylating agents. *J Biol Chem* 2001; **276**: 18193-18199 [PMID: 11279192 DOI: 10.1074/jbc.M101298200]

61 **Horton JK**, Wilson SH. Hypersensitivity phenotypes associated with genetic and synthetic inhibitor-induced base excision repair deficiency. *DNA Repair (Amst)* 2007; **6**: 530-543 [PMID: 17113833 DOI: 10.1016/j.dnarep.2006.10.016]

62 **Hu HY**, Horton JK, Gryk MR, Prasad R, Naron JM, Sun DA, Hecht SM, Wilson SH, Mullen GP. Identification of small molecule synthetic inhibitors of DNA polymerase beta by NMR chemical shift mapping. *J Biol Chem* 2004; **279**: 39736-39744 [PMID: 15258144 DOI: 10.1074/jbc.M402842200]

63 **Beard WA**, Wilson SH. Structure and mechanism of DNA polymerase Beta. *Chem Rev* 2006; **106**: 361-382 [PMID: 16464010 DOI: 10.1021/cr0404904]

P- Reviewer: Dhawan DK **S- Editor**: Zhai HH
L- Editor: Stewart GJ **E- Editor**: Zhang DN

WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7389

World J Gastroenterol 2013 November 14; 19(42): 7389-7398
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

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ORIGINAL ARTICLE

Radiofrequency ablation of hepatocellular carcinoma sized > 3 and ⩽ 5 cm: Is ablative margin of more than 1 cm justified?

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Supported by National Natural Science Foundation of China, No. 30872490 and 81172320; Dr. Wu Jie-Ping Medical Foundation, No. 320675012712 and 320675007131

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Received: June 29, 2013 **Revised:** September 10, 2013
Accepted: September 29, 2013

Published online: November 14, 2013

Abstract

AIM: To investigate whether an ablative margin (AM) > 1.0 cm might reduce chance of recurrence for patients with hepatocellular carcinoma (HCC) tumors 3.1 to 5.0 cm in size, compared with an AM of 0.5-1.0 cm.

METHODS: From October 2005 to December 2012, 936 consecutive patients with HCC who received radiofrequency ablation were screened. Of these, 281 patients, each with a single primary HCC tumor of 3.1 to 5.0 cm in size on its greatest diameter, were included in the study. Based on the AM width, we categorized patients into the 0.5-1.0 cm group and the > 1.0 cm group. Local tumor progression (LTP)-free survival, intrahepatic distant recurrence (IDR)-free survival and overall survival (OS) rates were obtained using the Kaplan-Meier method.

RESULTS: The 1-, 2-, 3-, 4-, and 5-year LTP-free survival rates and IDR-free survival rates were significantly higher in the > 1.0 cm group compared with the 0.5-1.0 cm group (97.5%, 86.3%, 73.6%, 49.5% and 26.4% *vs* 91.3%, 78.4%, 49.5%, 27.8%, and 12.8%; 95.1%, 90.3%, 77.0%, 61.0% and 48.3% *vs* 95.2%, 85.9%, 62.6%, 47.2% and 28.5%; $P < 0.05$). The 1-, 2-, 3-, 4-, and 5-year OS rates were 98.6%, 91.5%, 69.2%, 56.0% and 42.2%, respectively, in the 0.5-1.0 cm group and 100%, 98.9%, 90.1%, 68.7% and 57.4%, respectively, in the > 1.0 cm group ($P = 0.010$). There were no significant differences in complication rates between the two groups. Both univariate and multivariate analyses identified AM as an independent prognostic factor linked to LTP, IDR, and OS.

CONCLUSION: For HCC tumors > 3.0 cm and ⩽ 5.0 cm, AM > 1.0 cm could reduce chances of recurrence compared with AM of 0.5-1.0 cm, emphasizing the need for a more defensive strategy using AMs > 1.0 cm for ablating HCC tumors of 3.1 to 5.0 cm.

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Key words: Hepatocellular carcinoma; Radiofrequency ablation; Ablative margin; Recurrence; Survival

Core tip: Recurrence is the most important factor for prognosis of hepatocellular carcinoma (HCC) after radiofrequency ablation. Although a sufficient ablative margin (AM) is an essential way to minimize recurrence risk, the optimal AM for HCC tumors 3.1 to 5.0 cm remains controversial. This study provides evidence

that, for HCC tumors 3.1 to 5.0 cm, AMs > 1.0 cm could reduce chance of recurrence compared to AMs of 0.5-1.0 cm, which emphasizes the need for more strategic AMs that are > 1.0 cm for ablation of HCC tumors of 3.1 to 5.0 cm.

Ke S, Ding XM, Qian XJ, Zhou YM, Cao BX, Gao K, Sun WB. Radiofrequency ablation of hepatocellular carcinoma sized > 3 and ≤ 5 cm: Is ablative margin of more than 1 cm justified? *World J Gastroenterol* 2013; 19(42): 7389-7398 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7389.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7389

INTRODUCTION

Radiofrequency (RF) ablation is accepted as a potentially curative treatment modality for hepatocellular carcinoma (HCC) at an early stage when transplantation and resection are precluded[1,2]. Local tumor progression (LTP) and intrahepatic distant recurrence (IDR) have been repeatedly found to be the most important prognostic factors, with the incidence rates of 2%-53% and 43%-53%, respectively[1,3-5]. LTP that occurs after complete RF ablation is widely considered to result from residual tumor cells in the peritumoral area, which contains microvascular invasion and satellite micronodules due to an insufficient ablative margin (AM), although there is a possibility of *de novo* occurrence at the site. Pathogenesis of IDR is thought to result from intrahepatic metastasis of the primary tumor, viable residual tumor or an HCC of multicentric origin[6,7]. Therefore, a sufficient AM that encompasses both the main tumor and the area of adjacent parenchyma containing microvascular invasion or satellite micronodules, should theoretically ensure pathologically complete ablation, and would thus be an essential way to minimize risk of LTP and IDR[8,9].

The optimal safe AM for HCC is controversial[3,10-12]. Accumulating data have demonstrated that recurrence rates, such as LTP rates, differ greatly for tumors of various sizes but similar AMs[3,13]. For HCCs ≤ 3.0 cm, the 3-year LTP rates for patients treated by RF ablation with AMs of 0.5-1.0 cm are reportedly 10%-20%[3,14-17]. However, for HCC tumors 3.1 to 5.0 cm treated with AMs of 0.5-1.0 cm, the 3-year LTP rates were as high as 39%[18]. This can be well explained by the data from histopathological investigations on both the scope of peritumoral microvascular invasion and satellite micronodules and the incidence rate between HCCs ≤ 3.0 cm and those > 3.0 cm[19,20]. Only 14.5%-19% of single small HCC (≤ 3.0 cm) reportedly have satellite micronodules, located within 1.0 cm from the main tumor[21]. In contrast, for HCC tumors 3.1 to 5.0 cm, 26.3%-36.9% had peritumoral satellite micronodules, located more than 1.0 cm from the main tumor in most cases[9]. These data indicate that for HCCs ≤ 3.0 cm, AMs of 0.5-1.0 cm are likely to remove most peritumoral lesions. However, for HCC tumors 3.1 to 5.0 cm, AMs of ≤ 1.0 cm seem insufficient to ensure pathological complete tumor clearance in most cases.

Although the idea that AMs > 1.0 cm would be more likely to completely delete tumor tissues seems intuitively logical, no study to date has been conducted to determine the optimal AM for HCC tumors 3.1 to 5.0 cm. Based on the experience of the surgical requirement of a tumor-free margin ≥ 1.0 cm wide[9], we supposed that for HCC tumors 3.1 to 5.0 cm, AMs > 1.0 cm might reduce chance of recurrence compared with AMs of 0.5-1.0 cm. Therefore, the purpose of this study was to elucidate the survival benefit of AMs wider than 1.0 cm for HCC tumors 3.1 to 5.0 cm.

MATERIALS AND METHODS

Patients

To determine whether AMs >1.0 cm in RF ablation for HCC tumors 3.1 to 5.0 cm might greatly reduce chance of recurrence compared with AMs of 0.5-1.0 cm, a prospective cohort study was performed. From October 2005 to December 2012, 936 consecutive patients with HCC received RF ablation at the Department of Hepatobiliary Surgery, Beijing Chao-yang Hospital Affiliated to Capital Medical University, China. Among them, 327 patients suffered from a single primary HCC tumor each, 3.1 to 5.0 cm in diameter. Written informed consent was obtained from all patients. The study was approved by the investigation and ethics committee of Beijing Chao-yang Hospital, Capital Medical University according to the standards of the Declaration of Helsinki.

Among the 327 HCC patients, 291 who met the inclusion criteria were enrolled in this study. The inclusion criteria were (1) a single primary HCC tumor 3.1 to 5.0 cm at its greatest diameter on preoperative investigations; (2) no other therapy prior to RF ablation except for trans-arterial chemoembolization (TACE); (3) getting imaging-complete ablation with AM ≥ 0.5 cm after RF ablation; (4) no other therapy in the period of follow-up except for RF ablation or TACE for LTP or IDR; and (5) complete follow-up data. The exclusion criteria were (1) extrahepatic metastasis before LTP or IDR; and (2) follow-up period less than 6 mo. A total of 10 patients were excluded from the study for developing extrahepatic metastasis before LTP or IDR (*n* = 7), or follow-up period less than 6 mo (*n* = 3). The remaining 281 patients were included in this study (Table 1). There were 195 men and 86 women. Their median age was 52 years (range, 24-88 years). Of these, 231 (82.2%) were positive for serum hepatitis B virus surface antigen, 18 (6.4%) positive for serum HCV antibody, and 2 (0.7%) positive for both. Liver cirrhosis was observed in 92 patients (32.4%). In 126 of the 281 patients, preoperative diagnosis of HCC was histologically confirmed by needle biopsy under CT guidance. In the remaining 155 patients, HCC was established on the basis of compatible radiological features in contrast-enhanced multiphase helical CT scan and dynamic contrast-enhanced MRI. The median diameter of

Table 1 Comparison of patient clinical characteristics between the 0.5-1.0 cm group and the > 1.0 cm group *n* (%)

Variable	0.5-1.0 cm group (*n* = 158)	> 1.0 cm group (*n* = 123)	*P* value[1]
Age (yr)	52 (24-88)	51 (27-84)	0.467
Gender			
Male/female	109 (69.0)/49 (31.0)	86 (69.9)/37 (30.1)	0.873
Pre-existing hepatitis			
Hepatitis B	125 (79.1)	106 (86.2)	0.133
Hepatitis C	13 (8.2)	5 (4.1)	0.161
Hepatitis B and C	1 (0.6)	1 (0.8)	0.859
Child-Pugh grade			
Class A/class B	78 (49.4)/80 (50.6)	58 (47.2)/65 (52.8)	0.754
Liver cirrhosis			
Yes/No	55 (34.8)/103 (65.2)	37 (30.1)/86 (69.9)	0.417
Serum a-fetoprotein level (ng/mL)			
< 20	17 (10.8)	11 (8.9)	0.622
20-200	116 (73.4)	93 (75.6)	0.681
> 200	25 (15.8)	19 (15.4)	0.938
Tumor location			
S2,S3,S4,S6,S7/S5,S8	73 (46.2)/85 (53.8)	56 (45.5)/67 (54.5)	0.923
Biochemical analysis			
AST (IU/L)	46.5 (11.3-235.2)	46.1 (12.1-202.7)	0.793
ALT (IU/L)	48.4 (15.6-240.0)	47.9 (13.8-212.4)	0.560
Alb (g/dL)	3.7 (2.9-4.2)	3.6 (2.8-4.2)	0.460
T-Bil (mg/dL)	0.9 (0.7-1.4)	0.9 (0.6-1.4)	0.594
ALP (IU/L)	83.5 (7.7-376.6)	84.2 (8.1-284.8)	0.744
PT (%)	80.5 (58-100)	83.0 (59-100)	0.262
AFP (ng/mL)	83.2 (6.3-1301.9)	82.5 (5.4-750.7)	0.849
Tumor diameter(cm)	4.1 (3.1-5.0)	4.1 (3.3-5.0)	0.652
No. of ablation sessions before getting AM ≥ 0.5 cm			
1 session/2 sessions	115 (72.8)/43 (27.2)	99 (80.5)/24 (19.5)	0.143
Approaches of the first ablation session			0.978
Percutaneous	121 (76.6)	94 (76.4)	
Laparoscopic	37 (23.4)	29 (23.6)	
Open	0 (0)	0 (0)	

Values presented as absolute numbers (percent of cases) or median (range). [1]Fisher's exact test or Mann-Whitney *U* test. AFP: α-fetoprotein; Alb: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AM: Ablative margin; AST: Aspartate aminotransferase; PT: Prothrombin time; T-Bil: Total bilirubin.

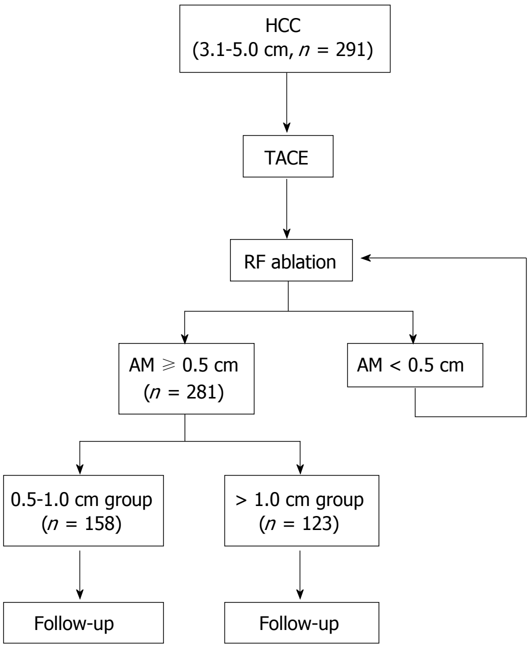

Figure 1 Flow diagram of the enrollment and follow-up. A total of 281 patients met the inclusion criteria. HCC: Hepatocellular carcinoma; RF: Radiofrequency; TACE: transcatheter arterial chemoembolization; AM: Ablative margin.

the HCC nodules was 4.1 cm (range: 3.1-5.0 cm). Tumor location was described according to Couinaud segmental anatomic classification. Liver function was classified according to the Child-Pugh classification. In all, 136 (48.4%) and 145 (51.6%) showed Child-Pugh classes A and B, respectively. Randomization was not performed in this study. The aim of this study was explained to all of the approved patients in advance, and safe AMs > 1.0 cm were tried in all patients, although AMs of ⩾ 0.5 cm are routinely considered adequate. On the basis of their AMs, we categorized patients into 2 groups: the 0.5-1.0 cm group and the > 1.0 cm group. Their demographic characteristics are shown in Table 1. The treatment algorithm of the present study is depicted in Figure 1.

TACE

In the study, TACE was performed in all patients, both for radiological assessment of AMs, and for oncological purposes, 2-3 wk before RF ablation by two interventional radiologists (YM Zhou and K Gao) with a standard regimen. TACE was performed through the femoral artery using the technique of Seldinger under local anesthesia[22] by injecting 6-10 mL of an emulsion of iodized oil (Lipiodol; Aulnay-Sous-Bois, France) and 20-40 mg of epirubicin hydrochloride (Zhejiang Haizheng, China) into the tumor feeding arteries. The selected doses of iodized oil and anticancer drug were individually based on the patient's liver function and tumor size. Injection was discontinued upon full accumulation of iodized oil into the tumor vessels. No gelatin sponge was used after TACE.

RF ablation

A percutaneous approach was most commonly preferred in this study. Laparoscopic RF ablation was considered in the presence of the following: (1) tumors on the liver edge or surface, protruding out of the liver; (2) tumors located in the left lobe, under the bottom of the heart, for which percutaneous RF ablation might cause heart injury; or (3) tumors close to visceral organs, such as the gallbladder, small and large bowels, and stomach. If other intra-abdominal procedures were planned, then open RF ablation was used.

Percutaneous RF ablation was performed under CT guidance (GE Yokogawa Medical Systems Ltd, Tokyo, Japan). To ensure the safety and tolerance of patients, each patient had respiratory control with a tracheal tube or a laryngeal mask airway under intravenous anesthesia during RF ablation procedure. This relatively more invasive protocol also helped improve targeting accuracy by providing a transient stop of respiration at end expiratory status during the procedures of planning and

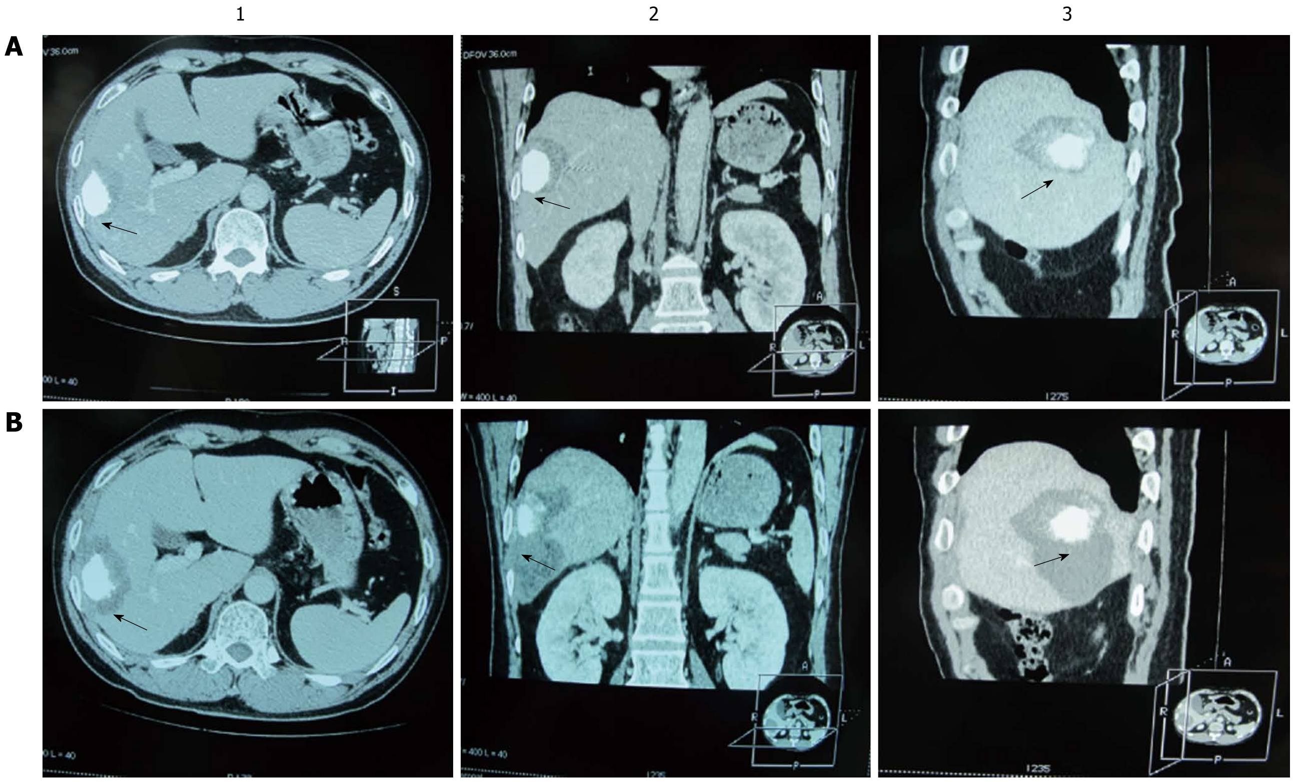

Figure 2 Three-dimensional computed tomography for radiofrequency ablation imaging in a patient with hepatocellular carcinoma. A: The computed tomography (CT) images prior to the second radiofrequency (RF) ablation; B: The CT images after the second RF ablation. 1: Axial plane; 2: Coronal plane; 3: Sagittal plane. The ablative margin is < 0.5 cm prior to the second RF ablation, and it has been ablated to ≥ 0.5 cm after the second RF ablation. Black arrow indicates the shortest width of the area of low density outside the iodine stained tumor.

targeting. Laparoscopic RF ablation and open RF ablation were performed with laparoscopic and open surgery techniques as usual with laparoscopic and intraoperative ultrasonographic assistance. All RF procedures in this study were performed using either a 15-gauge multitined electrode (Starburst XL; RITA Medical Systems, Manchester, GA, United States) or Cool-tip ACTC2025 or ACTC1525 electrodes, and an RF generator (RITA 1500; RITA Medical Systems Inc, Manchester, GA, United States or Covidien Healthcare, Ireland), according to their respective manufacturers' protocols. For patients treated with multitined expandable electrodes, when needle arrays are introduced into the tumor and positioned satisfactorily, the RF generator produces RF energy and maintains an average temperature of 105°C. A series of arrays, radiating from the central hollow probe, are pushed forward and unfolded gradually to 3, 4, or 5 cm until they reach the borders of the tumor. RF energy is delivered at 5-min intervals until the output power drops below 30 W in the final step of the procedure. For patients treated with Cool-tip electrodes, the RF generator (Covidien Healthcare, Ireland) was used. Unlike the RITA electrode, the Cool-tip electrode is straight, without arrays. With a 2.5 cm exposed tip, the Cool-tip electrodes can produce ablation zones of 4.5 cm with a single placement of electrodes and a maximum power of 200 W. Also, the Cool-tip RF generator continuously monitors tissue impedance throughout the procedure and adjusts the output accordingly. For this application, the ablation protocol was preset to the automatic mode, and ablation usually was carried out for 15 to 20 min, which was a little bit longer than that suggested in the manufacturer's protocol, with the intent of attaining a satisfactory degree of tumor collapse. Cold saline continuous irrigation of the needle was provided with an external pump. In the clinical settings, we selected the type of electrode depending on the size, geometry and location of the index tumor. A multiple-overlapping ablation technique was adopted for pursuing AMs ≥ 0.5 cm. Electrode track ablation technique was also performed to minimize post-procedural bleeding and tumor seeding.

Post-treatment assessment

For post-treatment evaluation, contrast-enhanced CT scans were performed 1 mo after the procedure in all cases. The initial unenhanced CT acquisition was followed by enhanced acquisitions obtained at 30 and 70 s after the bolus administration of intravenous contrast material. The contrast agent used was 100 mL iopamidol (Iopromide Injection, Bayer Schering Pharma, Guangzhou, China). Any contrast-enhancing areas beyond the margin of the ablation zone on post-ablation CT indicated incomplete tumor ablation. Additional sessions were scheduled for ablation of residual tumors. The diagnosis and treatment procedures were repeated until imaging

Table 2 Comparison of the recurrence pattern between the 0.5-1.0 cm group and > 1.0 cm group *n* (%)

Recurrence pattern	0.5-1.0 cm group (*n* = 158)	> 1.0 cm group (*n* = 123)	*P* value[1]
LTP only	74 (46.8)	39 (31.7)	0.01
IDR only	34 (21.5)	22 (17.9)	0.45
LTP + IDR	38 (24.1)	16 (13.0)	0.02
Total LTP	112 (70.9)	55 (44.7)	< 0.001
Total IDR	72 (45.6)	38 (30.9)	0.012

[1]Fisher's exact test. IDR: Intrahepatic distant recurrence; LTP: Local tumor progression.

complete ablation was achieved (Figure 1).

Evaluation of ablative margin

Three-dimensional reconstructions of CT images were made before and after RF ablation (Figure 2). To define the AM as accurate as possible, we performed qualitative side-by-side comparison of CT scans obtained before and after RF ablation. Two radiologists (Cao BX and Qian XJ) who were blind to the results of quantitative analysis assessed in consensus whether an AM of 0.5-1.0 cm or > 1.0 cm was achieved in each case. For this analysis, the adjacent hepatic vessels or the hepatic capsule were used to facilitate comparison. The variation between the two radiologists' findings was < 5%. In the evaluation, the AM was defined as the narrowest width of the area of low density outside the iodine stain (Figure 2). When the iodine stain in the tumor was not uniform, measuring AM was more difficult. In such instances, when the radiologists did not concur (n = 11), we compared carefully the imaging data before and after RF ablation, outlined the contours of the tumor in this area and measured AM according to the tumor contour rather than the edge of the iodine stain.

Follow-up

The follow-up protocol mainly included routine physical examination, laboratory tests, and measurement of AFP levels every month, as well as dynamic CT studies every 2 or 3 mo. Definitions are based on the standardization by the International Working Group on Image-Guided Tumor Ablation[23]. LTP was defined by the presence of a nodular lesion that was enhanced during the hepatic arterial phase and washed out by the delayed phase, and was found along the peripheral margin of the low-attenuated ablative zone. IDR was defined by a lesion with similar characteristics but not in contact with the original ablation zone in the liver. Overall survival (OS), defined as the interval between date of initial therapy and date of death or the last follow-up examination for living patients, was also evaluated.

In cases of LTP or IDR, other supplemental examinations like hepatic DSA, Lipiodol CT of the liver, CT of the chest and lower abdomen, and bone scintigraphy were performed for other potential tumor nodules. When LTP or IDR was confirmed, patients were hospitalized as soon as possible. Basically, repeat RF ablation treatment cycles were administered for LTP and IDR of ≤ 4 nodules. Five or more IDR nodules, or nodules in unsuitable locations for RF ablation, were treated with TACE. TACE was performed through the femoral artery using the aforementioned technique.

Complications

Major complications were assessed on the basis of the previously described guideline for image-guided tumor ablation[23]. Complication rates were evaluated for the total number of ablation sessions. We defined a major complication as an event that led to substantial morbidity and disability, increased the level of care required, resulted in hospital admission, or substantially lengthened the hospital stay. All other complications were considered minor.

Statistical analysis

Continuous data are expressed as median and range. Comparisons were made with the Mann-Whitney U test. Categorical data were compared using the Fisher's exact test. Rates of LTP-free survival, IDR-free survival and OS rates were calculated by the Kaplan-Meier method and compared using the log-rank test. Risk factors for LTP, IDR and overall survival were evaluated by univariate analyses using Cox regression tests. If multiple risk factors were shown to be significant by this test, we performed multivariate analysis using Cox regression tests to identify independent prognostic factors for LTP, IDR and OS. All statistical analyses were performed using the SPSS 15.0 statistical software (SPSS Inc., Chicago, Illinois, United States). All reported P values were 2-sided. $P < 0.05$ was considered statistically significant.

RESULTS

LTP-free survival

During the follow-up, LTP was found in 112 (70.9%) of 158 patients in the 0.5-1.0 cm group and in 55 (44.7%) of 123 patients in the > 1.0 cm group ($P < 0.001$) (Table 2). The rates of LTP-only and total LTP in the 0.5-1.0 cm group (46.8% and 70.9%, respectively) were significantly higher than those in the > 1.0 cm group (31.7% and 44.7%, respectively, P = 0.010 and < 0.001, respectively). The 1-, 2-, 3-, 4-, and 5-year LTP-free survival rates were 91.3%, 78.4%, 49.5%, 27.8% and 12.8%, respectively, in the 0.5-1.0 cm group and 97.5%, 86.3%, 73.6%, 49.5% and 26.4%, respectively, in the > 1.0 cm group (Figure 3A), and differed significantly between the two groups (P = 0.001).

IDR-free survival

During the follow-up, IDR was found in 72 (45.6%) of 158 patients in the 0.5-1.0 cm group and 38 (30.9%) of 123 patients in the > 1.0 cm group (Table 2). There

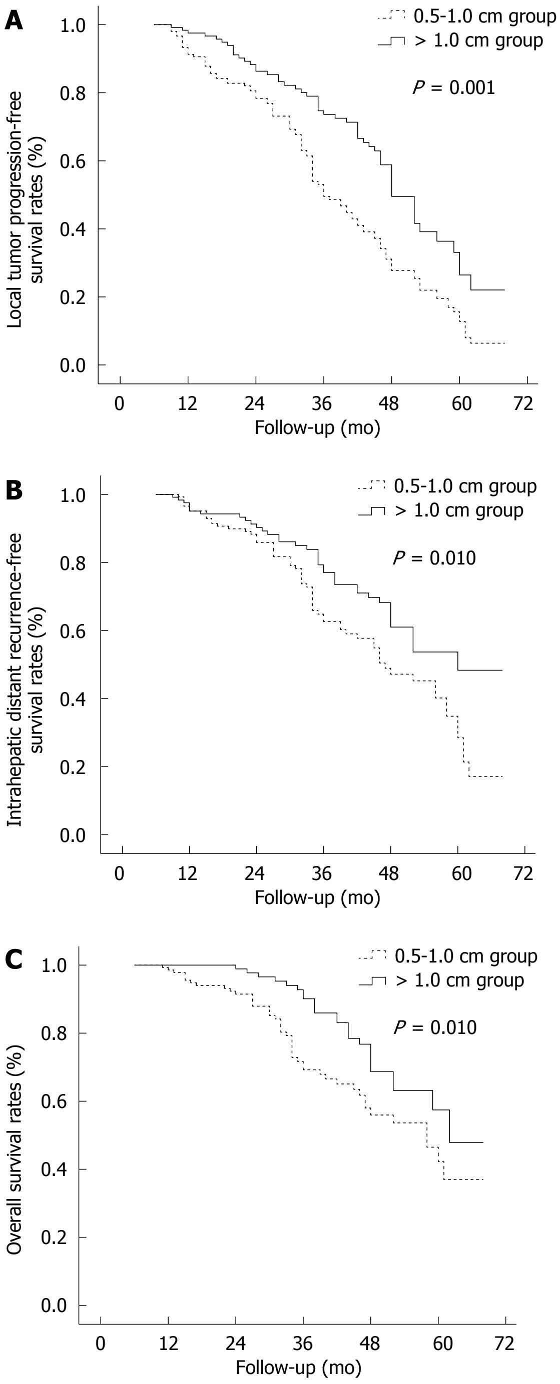

Figure 3 Kaplan-Meier analysis between the 0.5-1.0 cm group and the > 1.0 cm group. A: The local tumor progression-free survival rates; B: The intrahepatic distant recurrence-free survival rates; C: The overall survival rates.

was no significant difference in the rates of IDR-only between the 0.5-1.0 cm group and the > 1.0 cm group (21.5% *vs* 17.9%, P = 0.450). However, the rates of total IDR in the 0.5-1.0 cm group was significantly higher than those in the > 1.0 cm group (45.6% *vs* 30.9%, P = 0.012). The 1-, 2-, 3-, 4-, and 5-year IDR-free survival rates were 95.2%, 85.9%, 62.6%, 47.2% and 28.5%, respectively, in the 0.5-1.0 cm group and 95.1%, 90.3%, 77.0%, 61.0% and 48.3%, respectively, in the > 1.0 cm group (Figure 3B); the two groups differed statistically (P = 0.010).

OS rates

As of December 2012 (with a median follow-up of 38.2 mo), 203 patients (72.2%) remained alive, and 78 (27.8%) had died, including 55 patients in the 0.5-1.0 cm group and 23 patients in the > 1.0 cm group. The cause of death was HCC in 58 patients (74.4%), liver failure in 7 (9.0%), upper gastrointestinal bleeding in 3 (3.8%), causes unrelated to liver disease in 4 (including 3 patients who died of cardiovascular disease and 1 of cerebral hemorrhage; 5.1%), and undetermined causes in 6 patients (who died in emergency situations at other hospitals without definite diagnoses related to death; 7.7%). The 1-, 2-, 3-, 4-, and 5-year OS rates were 98.6%, 91.5%, 69.2%, 56.0% and 42.2%, respectively, in the 0.5-1.0 cm group and 100%, 98.9%, 90.1%, 68.7% and 57.4%, respectively, in the > 1.0 cm group (Figure 3C); the two groups differed significantly (P = 0.010, log-rank test).

Safety (complications)

No procedure-related death was observed. Major complications were observed in 5 (1.8%) of 281 patients. Of these, 2 patients in the 0.5-1.0 cm group and 1 patient in the > 1.0 cm group developed pneumothorax when laparoscopic RF ablation was performed. During the laparoscopic surgery, the presence of pneumothorax was confirmed immediately after the puncture of the RF probe. All of the pneumothorax was successfully treated by chest tube placement at the end of the operation. One patient in the 0.5-1.0 cm group suffered an intra-abdominal hemorrhage. He responded to transfusion of 2 units of packed red blood cells and required no other intervention. Two patients in the > 1.0 cm group developed hemopneumothorax and recovered from chest tightness and chest tube placement. Minor complications, including asymptomatic right pleural effusion, were noted within 3 d of the procedures in 13 patients of the 0.5-1.0 cm group and 15 patients of the > 1.0 cm group (0.5-1.0 cm group *vs* > 1.0 cm group, P = 0.271); however, none of these patients required interventional-drainage procedures.

Factors associated with LTP, IDR, and OS

Using univariate analysis, the tumor size (4.1-5.0 cm, P = 0.005), AM (> 1.0 cm, P = 0.003), AFP (> 200 ng/mL, P = 0.028), and number of ablation sessions for imaging complete ablation (CA, 2 sessions, P = 0.031) were found to be significant factors for predicting LTP, IDR, and OS (Table 3). In multivariate analyses of the 4 factors that were found to be significant in univariate analysis, the hazard ratios (HRs) for tumor size, AM, AFP, and number of ablation sessions for imaging CA are detailed in Table 4. Only the AM was found to be a significant independent factor linked to LTP, IDR, and OS.

Table 3 Significant variables in the univariate analysis for local tumor progression, intrahepatic distant recurrence, and overall survival (*n* = 281)

Significant variable	*n*	*P* value[1]		
		LTP	IDR	OS
Age (> 65 yr), yes/no	123/158	0.376	0.282	0.103
Gender (male), yes/no	195/76	0.571	0.436	0.854
Liver cirrhosis, yes/no	92/189	0.469	0.645	0.912
Child–Pugh grade (Class B), yes/no	145/136	0.173	0.742	0.811
Tumor location (S5,S8), yes/no	152/129	0.537	0.488	0.635
Tumor size (4.1-5.0 cm), yes/no	154/127	0.007	0.011	0.005
AM (> 1.0 cm), yes/no	123/158	0.001	0.024	0.003
AST (> 40 IU/L), yes/no	166/115	0.658	0.586	0.879
ALT (> 40 IU/L), yes/no	157/124	0.672	0.460	0.734
ALP (> 110 IU/L), yes/no	120/161	0.385	0.473	0.581
Alb (> 3.5 g/dL), yes/no	202/79	0.622	0.564	0.838
T-Bil (> 1 mg/dL), yes/no	93/188	0.541	0.502	0.796
PT (> 70%), yes/no	216/65	0.336	0.573	0.636
AFP (> 200 ng/mL), yes/no	44/237	0.016	0.032	0.028
Post-RF ablation antiviral therapy, yes/no	86/195	0.275	0.547	0.201
Body mass index (> 25 kg/m^2), yes/no	83/198	0.611	0.582	0.913
No. of ablation sessions before getting AM ≥ 0.5 cm (2 sessions), yes/no	67/214	0.034	0.041	0.031
Approaches of the first ablation session (Laparoscopic), yes/no	66/215	0.459	0.383	0.728

[1] Cox regression analysis. AFP: α-fetoprotein; Alb: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AM: Ablative margin; AST: Aspartate aminotransferase; IDR: Intrahepatic distant recurrence; LTP: Local tumor progression; OS: Overall survival; PT: Prothrombin time; T-Bil: Total bilirubin.

Table 4 Significant variables in the multivariate analysis for local tumor progression, intrahepatic distant recurrence, and overall survival (*n* = 281)

Significant variable	LTP			IDR			OS		
	HR	95%CI	*P* value[1]	HR	95%CI	*P* value[1]	HR ratio	95%CI	*P* value[1]
Tumor size (4.1-5.0 cm), yes/no	1.032	0.521-1.376	0.475	0.891	0.452-1.602	0.744	0.882	0.673-1.572	0.084
AM (> 1.0 cm), yes/no	1.484	0.101-1.812	0.001	1.278	1.137-1.729	0.025	1.604	0.881-2.753	0.002
AFP (> 200 ng/mL), yes/no	0.947	0.540-1.050	0.531	0.509	0.370-1.215	0.546	1.007	0.639-1.158	0.748
No. of ablation sessions before getting AM ≥ 0.5 cm (2 sessions), yes/no	1.012	0.683-1.772	0.663	0.923	0.562-1.218	0.347	0.745	0.321-.431	0.325

[1] Cox regression analysis. LTP: Local tumor progression; IDR: Intrahepatic distant recurrence; OS: Overall survival; AFP: α-fetoprotein; AM: Ablative margin.

DISCUSSION

Over the past years, the rapid development and refinement of RF ablation technology has led to increasing use of this treatment modality in HCC patients[24]. Extensive clinical studies support RF ablation as a preferred treatment for very early HCC[25]. However, tumor recurrence, including LTP and IDR, frequently occurs, affecting the prognosis. Furthermore, rapid tumor progression after RF ablation, which may mostly be associated with the progression of residual HCC, has been gaining increasing attention[26-29]. These experimental data indicated that any residual tumors might be a disaster for individual HCC patients who received RF ablation. Thus, we should try to remove microvascular invasions and satellite micronodules around the main tumor of HCC to decrease the likelihood of residual tumor, the incidence rates of LTP, IDR and the rapid tumor progression.

From perspective of pathological clearance of the tumor tissue, AMs should be as wide as possible. However, correctly obtaining a sufficient AM around all sides of a tumor of medium size is not easy. Specifically, to get a 1.0-cm AM for lesions sized 3.0, 4.0 and 5.0 cm in diameters, the volumes of peritumoral tissue to ablate are 3.6, 2.4 and 1.7 times, respectively, the volume of the main tumor[30]. A little increase of the dimension of the ablation zone means a tremendous increase of the amount of ablation tissue. For example, if we increase the AM from 1.0 to 1.5 cm for a lesion of 4.0 or 5.0 cm in diameter, respectively, the amounts of tissue to ablate will be theoretically increased by as much as 58.8% and 49.3% respectively, and this does not take into account the increased difficulty and time to accomplish it. So we should balance the perfect AM standard against its feasibility in clinical practice. As a compromise settlement, we take an AM of more than 1.0 cm as a minimum requirement for HCC nodules of 3.1 to 5.0 cm.

Although an AM > 1.0 cm cannot guarantee that all peritumoral tumor tissues are ablated in all cases, 2 ways can be used to compensate for the conservativeness of this standard. First, there may be some decrease in the size of the ablation zone due to tissue healing and scar formation when measured 1 mo after ablation and the real AM might be underestimated in some extent. Second, in our performance of RF ablation treatment, we took an AM of more than 1 cm thick as the least requirement and tried to make it as wide as possible.

The precise evaluation of AM after RF ablation is rather difficult, especially for HCC lesions of medium size or larger which did not get TACE/trans-arterial embolization (TAE) pretreatment[3]. First, AM area is not clearly visualized on images because both the ablated tumor and AM appear as areas that lack contrast enhancement. Second, AMs around tumors are usually not symmetrical and the measurement of AM by subtracting the diameter of the index tumor from that of ablation zone is not as precise as expected. So in the present study, all enrolled patients received TACE pretreatment, aiming to facilitate the evaluation of AM as precise as possible.

We demonstrated that tumor size, AM, AFP, and number of ablation sessions before getting an AM > 1.0 cm were significant risk factors for LTP, IDR, and OS of HCC after RF ablation, using univariate analysis. Our results were in line with those of previous studies on risk factors related to recurrence of HCC after RF ablation[11,31]. However, only AM was found to be a significant independent factor linked to LTP, IDR, and OS of HCC after RF ablation using multivariate analysis. These findings confirmed that AMs > 1.0 cm are an important predictive factor for recurrence of HCC tumors 3.1 to 5.0 cm after RF ablation. It is easy to understand that an AM >1.0 cm can reduce rates of LTP and IDR and eventually increase OS rate. An AM > 1.0 cm can lead to a further clearance of possible residues of microvascular invasion and satellite micronodules by ablating more viable tumor containing liver parenchyma and decrease probability of metastasis of the residual tumor cell by intrahepatic portal vein.

We are aware of the limitations of this analysis. This study was not randomized. Moreover, the AM standard of 1.0 cm referred only to the safety margin for liver resection advocated by most surgeons. Also, the biological nature of HCC as a commonly accepted important risk factor was not demonstrated in this study due to the limited number of cases with pathological examination. One further limitation is the fact that this was a single-center study; these results might not be reproducible consistently in other settings. The results may be influenced by the physicians' expertise and the institution's volume of care. Nevertheless, our data may be helpful for clinicians who treat HCC with RF ablation and may also be useful as a basis for the design of future trials. Again, more prospective, large randomized studies are needed to assess the benefit of AMs > 1.0 cm for medium-sized HCC lesions in patients who receive RF ablation.

Notwithstanding its preliminary character, this study does provide evidence that, for HCC tumors 3.1 to 5.0 cm, AMs > 1.0 cm could reduce chance of recurrence compared with AMs of 0.5-1.0 cm, which emphasizes the need for more defensive strategies in using AMs wider than 1.0 cm for ablation of HCC tumors 3.1 to 5.0 cm. However, confirmation in a prospective multicenter randomized trial is required.

COMMENTS

Background

Radiofrequency (RF) ablation is becoming accepted as a promising technique for treatment of patients with hepatocellular carcinoma (HCC). Recurrence of HCC after RF ablation occurs frequently and is associated with poor prognosis. A sufficient ablative ablative margin (AM) is an essential way to minimize the risk of recurrence. However, the optimal AM for HCC tumors 3.1 to 5.0 cm in size is unclear.

Research frontiers

Theoretically, AMs that encompass both the main tumor and an area of adjacent parenchyma that contains microvascular invasion or satellite micronodules, ensuring a pathological complete ablation, will minimize risk of tumor recurrence. A current research hotspot is to affirm that, for HCC tumors 3.1 to 5.0 cm, AMs > 1.0 cm reduce recurrence risk compared with AMs of 0.5-1.0 cm.

Innovations and breakthroughs

Accumulating data show that the recurrence rates after RF ablation, such as local tumor progression (LTP) or intrahepatic distant recurrence (IDR) rate, are quite different in varied-sized tumors with similar AMs. For HCC tumors ≤ 3.0 cm, an AM of 0.5-1.0 cm may remove most peritumoral lesions. However, for HCC tumors 3.1 to 5.0 cm, AMs ≤ 1.0 cm seem insufficient to ensure pathological complete tumor clearance in most cases. In this study, we confirmed that AMs > 1.0 cm are an important predictor for recurrence of HCC tumors 3.1 to 5.0 cm after RF ablation.

Applications

The study results provide evidence that, for HCC tumors 3.1 to 5.0 cm, AMs > 1.0 cm could reduce chances of recurrence compared with AMs of 0.5-1.0 cm, emphasizing the need for a more defensive strategy that used AMs > 1.0 cm for ablation of HCC tumors 3.1 to 5.0 cm.

Terminology

LTP was defined by the presence of a nodular lesion that was enhanced during the hepatic arterial phase and washed out by the delayed phase and was found along the peripheral margin of the low-attenuated ablative zone. IDR was defined by a lesion with similar characteristics that did not contact the original ablation zone in the liver. Overall survival was defined as the interval between date of initial therapy and date of death, or the last follow-up examination for living patients.

Peer review

This is a good prospective cohort study in which the authors analyzed the survival benefit of radiofrequency AMs > 1.0 cm for HCC tumors 3.1 to 5.0 cm. The results are interesting and suggest that for HCC tumors 3.1 to 5.0 cm, AMs > 1.0 cm could reduce chance of recurrence compared with narrower AMs of 0.5-1.0 cm, emphasizing the need for a more defensive strategy, using wider AMs > 1.0 cm for ablation of HCC tumors 3.1 to 5.0 cm.

REFERENCES

1 **Lau WY**, Lai EC. The current role of radiofrequency ablation in the management of hepatocellular carcinoma: a systematic review. *Ann Surg* 2009; **249**: 20-25 [PMID: 19106671 DOI: 10.1097/SLA.0b013e31818eec29]

2 **Lencioni R**. Loco-regional treatment of hepatocellular carcinoma. *Hepatology* 2010; **52**: 762-773 [PMID: 20564355 DOI: 10.1002/hep.23725]

3 **Nakazawa T**, Kokubu S, Shibuya A, Ono K, Watanabe M, Hidaka H, Tsuchihashi T, Saigenji K. Radiofrequency ablation of hepatocellular carcinoma: correlation between local

tumor progression after ablation and ablative margin. *AJR Am J Roentgenol* 2007; **188**: 480-488 [PMID: 17242258 DOI: 10.2214/AJR.05.2079]

4 **Livraghi T**. Radiofrequency ablation of hepatocellular carcinoma. *Surg Oncol Clin N Am* 2011; **20**: 281-99, viii [PMID: 21377584 DOI: 10.1016/j.soc.2010.11.010]

5 **Kim H**, Rhim H, Choi D, Lim HK, Kim YS, Lee WJ, Joh JW. Recurrence and treatment pattern in long-term survivors with hepatocellular carcinoma: a comparison between radiofrequency ablation and surgery as a first-line treatment. *World J Surg* 2010; **34**: 1881-1886 [PMID: 20376449 DOI: 10.1007/s00268-010-0533-1]

6 **Sumie S**, Kuromatsu R, Okuda K, Ando E, Takata A, Fukushima N, Watanabe Y, Kojiro M, Sata M. Microvascular invasion in patients with hepatocellular carcinoma and its predictable clinicopathological factors. *Ann Surg Oncol* 2008; **15**: 1375-1382 [PMID: 18324443 DOI: 10.1245/s10434-008-9846-9]

7 **Zytoon AA**, Ishii H, Murakami K, El-Kholy MR, Furuse J, El-Dorry A, El-Malah A. Recurrence-free survival after radiofrequency ablation of hepatocellular carcinoma. A registry report of the impact of risk factors on outcome. *Jpn J Clin Oncol* 2007; **37**: 658-672 [PMID: 17766723 DOI: 10.1093/jjco/hym086]

8 **Shindoh J**, Hasegawa K, Inoue Y, Ishizawa T, Nagata R, Aoki T, Sakamoto Y, Sugawara Y, Makuuchi M, Kokudo N. Risk factors of post-operative recurrence and adequate surgical approach to improve long-term outcomes of hepatocellular carcinoma. *HPB (Oxford)* 2013; **15**: 31-39 [PMID: 23216777 DOI: 10.1111/j.1477-2574.2012.00552.x]

9 **Shi M**, Zhang CQ, Zhang YQ, Liang XM, Li JQ. Micrometastases of solitary hepatocellular carcinoma and appropriate resection margin. *World J Surg* 2004; **28**: 376-381 [PMID: 15022021 DOI: 10.1007/s00268-003-7308-x]

10 **Kim YS**, Lee WJ, Rhim H, Lim HK, Choi D, Lee JY. The minimal ablative margin of radiofrequency ablation of hepatocellular carcinoma (& gt; 2 and & lt; 5 cm) needed to prevent local tumor progression: 3D quantitative assessment using CT image fusion. *AJR Am J Roentgenol* 2010; **195**: 758-765 [PMID: 20729457 DOI: 10.2214/AJR.09.2954]

11 **Kim YS**, Rhim H, Cho OK, Koh BH, Kim Y. Intrahepatic recurrence after percutaneous radiofrequency ablation of hepatocellular carcinoma: analysis of the pattern and risk factors. *Eur J Radiol* 2006; **59**: 432-441 [PMID: 16690240 DOI: 10.1016/j.ejrad.2006.03.007]

12 **Nishikawa H**, Inuzuka T, Takeda H, Nakajima J, Sakamoto A, Henmi S, Matsuda F, Eso Y, Ishikawa T, Saito S, Kita R, Kimura T, Osaki Y. Percutaneous radiofrequency ablation therapy for hepatocellular carcinoma: a proposed new grading system for the ablative margin and prediction of local tumor progression and its validation. *J Gastroenterol* 2011; **46**: 1418-1426 [PMID: 21845378 DOI: 10.1007/s00535-011-0452-4]

13 **Kim JH**, Won HJ, Shin YM, Kim SH, Yoon HK, Sung KB, Kim PN. Medium-sized (3.1-5.0 cm) hepatocellular carcinoma: transarterial chemoembolization plus radiofrequency ablation versus radiofrequency ablation alone. *Ann Surg Oncol* 2011; **18**: 1624-1629 [PMID: 21445671 DOI: 10.1245/s10434-011-1673-8]

14 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54**: 1151-1156 [PMID: 16009687 DOI: 10.1136/gut.2004.045203]

15 **Shiina S**, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122-130 [PMID: 16012942 DOI: 10.1053/j.gastro.2005.04.009]

16 **Tateishi R**, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005; **103**: 1201-1209 [PMID: 15690326 DOI: 10.1002/cncr.20892]

17 **Choi D**, Lim HK, Rhim H, Kim YS, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Yoo BC. Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. *Eur Radiol* 2007; **17**: 684-692 [PMID: 17093964 DOI: 10.1007/s00330-006-0461-5]

18 **Morimoto M**, Numata K, Kondou M, Nozaki A, Morita S, Tanaka K. Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: a randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer* 2010; **116**: 5452-5460 [PMID: 20672352 DOI: 10.1002/cncr.25314]

19 **Okusaka T**, Okada S, Ueno H, Ikeda M, Shimada K, Yamamoto J, Kosuge T, Yamasaki S, Fukushima N, Sakamoto M. Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. *Cancer* 2002; **95**: 1931-1937 [PMID: 12404287 DOI: 10.1002/cncr.10892]

20 **McHugh PP**, Gilbert J, Vera S, Koch A, Ranjan D, Gedaly R. Alpha-fetoprotein and tumour size are associated with microvascular invasion in explanted livers of patients undergoing transplantation with hepatocellular carcinoma. *HPB (Oxford)* 2010; **12**: 56-61 [PMID: 20495646 DOI: 10.1111/j.1477-2574.2009.00128.x]

21 **Ikeda K**, Seki T, Umehara H, Inokuchi R, Tamai T, Sakaida N, Uemura Y, Kamiyama Y, Okazaki K. Clinicopathologic study of small hepatocellular carcinoma with microscopic satellite nodules to determine the extent of tumor ablation by local therapy. *Int J Oncol* 2007; **31**: 485-491 [PMID: 17671673]

22 **Matsui O**, Kadoya M, Yoshikawa J, Gabata T, Takashima T, Demachi H. Subsegmental transcatheter arterial embolization for small hepatocellular carcinomas: local therapeutic effect and 5-year survival rate. *Cancer Chemother Pharmacol* 1994; **33** Suppl: S84-S88 [PMID: 8137490]

23 **Goldberg SN**, Grassi CJ, Cardella JF, Charboneau JW, Dodd GD, Dupuy DE, Gervais DA, Gillams AR, Kane RA, Lee FT, Livraghi T, McGahan J, Phillips DA, Rhim H, Silverman SG, Solbiati L, Vogl TJ, Wood BJ, Vedantham S, Sacks D. Image-guided tumor ablation: standardization of terminology and reporting criteria. *J Vasc Interv Radiol* 2009; **20**: S377-S390 [PMID: 19560026 DOI: 10.1016/j.jvir.2009.04.011]

24 **Kim YS**, Lim HK, Rhim H, Lee MW, Choi D, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Gwak GY, Yoo BC. Ten-year outcomes of percutaneous radiofrequency ablation as first-line therapy of early hepatocellular carcinoma: analysis of prognostic factors. *J Hepatol* 2013; **58**: 89-97 [PMID: 23023009 DOI: 10.1016/j.jhep.2012.09.020]

25 **Chen MS**, Peng ZW, Xu L, Zhang YJ, Liang HH, Li JQ. Role of radiofrequency ablation in the treatment of hepatocellular carcinoma: experience of a cancer center in China. *Oncology* 2011; **81** Suppl 1: 100-104 [PMID: 22212942 DOI: 10.1159/000333268]

26 **Kong J**, Kong J, Pan B, Ke S, Dong S, Li X, Zhou A, Zheng L, Sun WB. Insufficient radiofrequency ablation promotes angiogenesis of residual hepatocellular carcinoma via HIF-1α/VEGFA. *PLoS One* 2012; **7**: e37266 [PMID: 22615958 DOI: 10.1371/journal.pone.0037266]

27 **Ke S**, Ding XM, Kong J, Gao J, Wang SH, Cheng Y, Sun WB. Low temperature of radiofrequency ablation at the target sites can facilitate rapid progression of residual hepatic VX2 carcinoma. *J Transl Med* 2010; **8**: 73 [PMID: 20667141 DOI: 10.1186/1479-5876-8-73]

28 **Kong J**, Kong L, Kong J, Ke S, Gao J, Ding X, Zheng L, Sun H, Sun W. After insufficient radiofrequency ablation, tumor-associated endothelial cells exhibit enhanced angiogenesis and

promote invasiveness of residual hepatocellular carcinoma. *J Transl Med* 2012; **10**: 230 [PMID: 23171368 DOI: 10.1186/1479-5876-10-230]

29 **Ruzzenente A**, Manzoni GD, Molfetta M, Pachera S, Genco B, Donataccio M, Guglielmi A. Rapid progression of hepatocellular carcinoma after Radiofrequency Ablation. *World J Gastroenterol* 2004; **10**: 1137-1140 [PMID: 15069713]

30 **Dodd GD**, Frank MS, Aribandi M, Chopra S, Chintapalli KN. Radiofrequency thermal ablation: computer analysis of the size of the thermal injury created by overlapping ablations. *AJR Am J Roentgenol* 2001; **177**: 777-782 [PMID: 11566672]

31 **Fernandes ML**, Lin CC, Lin CJ, Chen WT, Lin SM. Risk of tumour progression in early-stage hepatocellular carcinoma after radiofrequency ablation. *Br J Surg* 2009; **96**: 756-762 [PMID: 19526609 DOI: 10.1002/bjs.6645]

P- Reviewers: Cucchetti A, Radu A, Xu HX **S- Editor**: Ma YJ
L- Editor: Wang TQ **E- Editor**: Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7399

World J Gastroenterol 2013 November 14; 19(42): 7399-7404
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Interleukin 28B-related polymorphisms: A pathway for understanding hepatitis C virus infection?

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Supported by Grants from the Research Fund from University of Region of Joinville, FAP-UNIVILLE

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Received: January 21, 2013 **Revised:** August 19, 2013
Accepted: September 4, 2013
Published online: November 14, 2013

Abstract

AIM: To analyze the role of *rs12979860* and *rs8099917* polymorphisms in hepatitis C virus (HCV) genotype 1 infection of Brazilians.

METHODS: A total of 145 adult patients diagnosed with genotype 1 chronic hepatitis C (CHC) who had completed a 48-wk regimen of pegylated-interferon α-2a or -2b plus ribavirin combination therapy were recruited from six large urban healthcare centers and 199 healthy blood donors (controls) from a single site between January 2010 and January 2012. Data on the patients' response to treatment was collected. Polymerase chain reaction-restriction fragment length polymorphism genotyping of the interleukin (*IL*)*28B* gene fragment encompassing the single nucleotide polymorphisms (SNPs) rs12979860 (C/T) and rs8099917 (T/G) was carried out for 79 of the CHC patients and 199 of the controls. Bi-directional amplicon sequencing of the two SNPs was carried out for the remaining 66 CHC patients.

RESULTS: SNP rs12979860 genotyping was successful in 99.5% of the controls and 97.2% of the CHC patients, whereas the SNP rs8099917 genotyping was successful in 95.5% of the controls and 100% of the

CHC patients. The genotype and allele distributions for both rs12979860 and rs8099917 were significantly different between the control and CHC patient groups, with significantly higher genotype frequencies of CC and TT in the controls (P = 0.037 and 0.046, respectively) and of TT and GG in the CHC patients (P = 0.0009 and 0.0001, respectively). Analysis of the CHC patients who achieved sustained virological response (SVR) to treatment (n = 55) indicated that the rs12979860 C allele and CC genotype were predictors of SVR (P = 0.02). No significant correlation was found between rs8099917 genotypes and treatment response, but carriers of the T allele showed significantly higher rates of SVR (P = 0.02). Linkage disequilibrium analysis of the group that achieved SVR showed a significant association between rs12979860 and rs8099917 (P = 0.07).

CONCLUSION: The higher allele frequency of rs12979860 C and rs8099917 T observed in non-HCV-infected individuals may indicate a potential protective role for these *IL28B*-related polymorphisms.

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Key words: Hepatitis C; Interleukin 28B; Single nucleotide polymorphisms; Sustained virological response; Brazil

Core tip: This study investigated the differential distribution of interleukin 28B genetic variants between patients with chronic hepatitis C genotype 1 infection and non-infected healthy controls, and evaluated the association of these polymorphisms with patient response to standard antiviral therapeutic regimens. Genotype and allele frequencies of rs12979860 and rs8099917 were significantly different between the patients and controls, and the patterns suggested a potential protective role against hepatitis C virus infection. Finally, the rs12979860 CC genotype showed correlation to achievement of sustained virological response following pegylated-interferon/ribavirin-based therapy.

Garcia RFL, Moreira S, Ramos ALA, Ferreira LE, Mattos AA, Tovo CV, Nader LA, Ramos JA, Rondinelli E, Dominici AJ, Garcia CE, Pinho MSL, Brandão-Mello CE, Villela-Nogueira CA, França PHC. Interleukin 28B-related polymorphisms: A pathway for understanding hepatitis C virus infection? *World J Gastroenterol* 2013; 19(42): 7399-7404 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7399.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7399

INTRODUCTION

Hepatitis C virus (HCV) infection results in a broad spectrum of clinical outcomes and symptoms, ranging from spontaneous viral elimination to chronic infection and from jaundice to end-stage liver failure. Despite the introduction of highly efficacious antiviral drugs based on viral protease inhibition, most countries continue to rely on the established combination therapy based on an immune-modulator plus an antiviral [most frequently pegylated-interferon α-2a or -2b (PegIFN-α-2a/2b) and ribavirin (RBV)] even though this strategy is associated with high failure rates[1]. Studies to uncover the mechanisms underlying non-/low- or unsustained response to treatment have identified a number of contributing factors, related to both the virus itself (*i.e.*, genotype and viral load) and the host (*i.e.*, age, sex, ethnicity, concomitant liver fibrosis, and metabolic abnormalities[2,3]; thus, it is theorized that interaction between the virus and host genetics may be an important determinant of the natural history of HCV infection and may be predictive of therapy outcome.

A recent genome-wide association study (GWAS) of > 600000 polymorphisms in a cohort of HCV-infected patients found a single nucleotide polymorphism (SNP) on chromosome 19 associated with therapy response[4]. Subsequent GWAS studies implicated the *rs8099917* (TG) polymorphism in the genetic region of this chromosome related to the interleukin (*IL*)*28B* gene and showed direct associations of the SNP to spontaneous clearance of HCV and treatment outcome[5,6]. Subsequent related studies have identified an additional *IL28B*-related SNP, rs12979860, and confirmed these two polymorphisms as strong predictors of therapy response and contributors to the epidemiological and ethnical distribution of HCV infection[7-9]. In addition, comparative analysis has identified significant differential prevalence profiles of these SNPs for HCV-infected patients and healthy controls[4], suggesting potential roles for each in disease susceptibility and/or resistance.

The prevalence rate of HCV remains high in Brazil, particularly when compared to the other Latin American countries[10], yet little data is available on the genetic variation profile of this disease in Brazilians. This study was designed to analyze the differential genotypic and allelic distributions of the *IL28B*-related SNPs, rs12979860 and rs8099917, in a cohort of Brazilian patients infected with HCV genotype 1 and healthy controls, as well as to investigate the potential association of these polymorphisms with treatment outcome.

MATERIALS AND METHODS

Patients

Between January 2010 and January 2012, adult patients who had undergone combination therapy for chronic infection with HCV genotype 1 [48-wk combination of PegIFN-α-2a/2b and RBV (15 mg/kg)] were recruited from the following six large urban healthcare centers: Hospital Municipal São José, Joinville/SC (n = 25); Hospital Universitário Gaffrée Guinle (n = 26) and Hospital Universitário Clementino Fraga Filho (n = 66), Rio de Janeiro/RJ; Hospital da Universidade Federal de Pelotas (n = 12), Pelotas/RS; Santa Casa de Misericórdia (n = 2),

Table 1 Demographic characteristics of the study participants

Characteristic	HCV (*n* = 145)	Control (*n* = 198)
Age (yr), mean ± SD	55.5 ± 10.0	32.2 ± 10.0
Sex, M/F	52%/48%	66%/34%
Skin color		
White	75.70%	88.00%
Black	19.20%	5.00%
Brown	5.10%	7.00%

HCV: Hepatitis C virus.

Porto Alegre/RS; and Hospital da Universidade Federal do Maranhão (*n* = 14), São Luis/MA. Patients with co-infection of hepatitis B virus or human immunodeficiency virus, or with any other concomitant chronic liver disease, were denied study enrollment. Blood sample and data on treatment outcome was collected for each enrolled patient. The treatment outcome of sustained virological response (SVR) was defined by a negative result for detection of serum HCV RNA at month six post-treatment. Patients who did not achieve SVR (including those who relapsed) were collectively categorized as non-responders (NR).

Healthy controls

Healthy individuals presenting for blood donation at HEMOSC Joinville/SC in 2010 were recruited for the study. Study enrollment was offered to individuals with no clinical or laboratory evidence of liver disease or other major pathological conditions. All enrolled controls were > 18 years old and lacked familial relationship.

Racial classification

To phenotypically classify each study participant by race, we employed the criteria published by the Brazilian Institute of Geography and Statistics[11]; the skin color categories (white, brown, black, yellow, and Indian) were self-reported by each participant.

Ethics statement

The study was approved by each of the participating healthcare center's Institutional Committees, which required adherence to the Brazilian normative for ethics in research. Informed consent was obtained from each participant before blood sampling.

Genotyping methods

The HCV patients' blood samples were collected by finger puncture and stored as blots on FTA Elute Micro Cards® (Whatman, Kent, United Kingdom) for subsequent genomic DNA extraction according to the manufacturer's protocol. The controls' blood samples were collected by venipuncture and stored in EDTA for subsequent genomic DNA extraction *via* the QIAamp DNA Miniprep Kit (Qiagen, Chatsworth, CA, United States).

The genotyping of SNPs rs12979860 and rs8099917 was performed at the Universidade da Região de Joinville and the Instituto de Biofísica Carlos Chagas Filho. For all 199 control samples and 79 of the HCV-infected samples, genotyping was performed *via* PCR-restriction fragment length polymorphism analysis[12] on an LGC XP thermocycler (BIOER Technology Co., Tokyo, Japan) and results were documented by the Mini-Bis Pro Gel Imaging System (DNR Bio-Image Systems Ltd., Jerusalem, Israel). The remaining 66 HCV-infected samples were genotyped by bi-directional amplicon sequencing using the DYEnamic™ ET Dye Terminator Cycle Sequencing Kit and the MegaBACE 1000 DNA Sequencer (GE Healthcare, Pittsburgh, PA, United States). The HCV treatment responses and *IL28* genotyping results of this subgroup of patients were previously reported[13].

Statistical analysis

Data analysis was performed by the SPSS statistical software (v13.0; SPSS Inc., Chicago, IL, United States). Continuous variables were compared by Student's *t*-test for parametric variables, or by Mann-Whitney *U* test for non-parametric distributions. All *P*-values are two-tailed and values < 0.05 were considered statistically significant. The χ^2 G test for "Goodness of Fit" was used to verify whether the proportions of the polymorphisms were unequally distributed between controls and patients or in Hardy-Weinberg equilibrium (HWE). Differences in allele and genotype frequencies between different groups were assessed by Pearson's χ^2 test (with χ^2 test for linear trend when appropriate).

RESULTS

The demographic characteristics of the HCV-infected patients and healthy controls are summarized in Table 1. SNP rs12979860 genotyping was successful in 142 (97.2%) of the patients and 198 (99.5%) of the controls, and SNP rs8099917 genotyping was successful in all 145 (100%) of the patients and 190 (95.5%) of the controls.

Genotype and allele distributions among the HCV-infected patients and healthy controls

Analysis of rs12979860 showed that the HCV-infected group had a significantly lower prevalence of the CC genotype (30.3% *vs* control group: 47.4%, *P* = 0.037) and the C allele (0.53 *vs* 0.69, *P* = 0.0073) and T allele (0.47 *vs* 0.31, *P* = 0.0073), but a significantly higher prevalence of the TT genotype (24.8% *vs* 8.9%, *P* = 0.0009). Analysis of rs8099917 showed that the HCV-infected group had a significantly lower prevalence of the TT genotype (46.2% *vs* control group: 67.4%, *P* = 0.043) and of the T allele (0.63 *vs* 0.83, *P* = 0.0009), but a significantly higher prevalence of the GG genotype (20.0% *vs* 1.5%, *P* = 0.0001) and of the G allele (0.37 *vs* 0.17, *P* = 0.0008).

Both of the *IL28B* SNPs were in HWE among individuals belonging to the control group (rs12979860, *P* = 0.7; rs8099917, *P* = 0.1), but only the rs12979860 SNP was in HWE for the HCV group (*P* = 0.21). Linkage disequilibrium analysis showed that both SNPs were associated with the controls as well as the HCV-infected patients (*P* = 0.0001).

Table 2 Demographic characteristics and clinical parameters of hepatitis c virus-infected patients stratified by response to treatment

Feature	SVR (n = 55)	NR (n = 89)	P value
Sex, M/F	54%/46%	51%/49%	0.072
Skin color			
White	89%	70%	0.032
Black	11%	26%	0.009
Brown	-	4%	ND
ALT in UI/mL, mean (IQR)	97 (54)	77 (64)	0.1
Baseline viral load in UI/mL[1]			
< 600000	47.80%	49.30%	0.760
≥ 600000	52.20%	50.70%	0.890
Fibrosis score[2]			
0-2	63.30%	49.40%	0.125
3-4	36.70%	50.60%	0.126

[1]This parameter was only available for 115 patients [46 of sustained virological response (SVR), and 69 of non-responders (NR)]; [2]According to histopathological analysis of liver biopsy tissues; this parameter was only available for 128 patients (49 of SVR, and 79 of NR). ND: Not done due to small numbers of cases; ALT: Alanine aminotransferase.

Correlation between the IL28B polymorphisms and treatment outcomes

Fifty-five (39%) of the patients achieved SVR following the combination therapy. Stratification analysis of the SVR patients by race showed that the rate was significantly lower in the self-declared black individuals (P = 0.009). None of the clinical parameters were significantly different between the SVR and NR groups (Table 2). However, the SVR group did have significantly higher prevalences of the rs12979860 CC genotype (45.5% *vs* NR: 21.0%, P = 0.02) and C allele (0.64 *vs* 0.46, P = 0.019). Conversely, the prevalences of the rs12979860 TT genotype and T allele were significantly higher in the NR group (30.0% *vs* SVR: 18.2%, P = 0.03 and 0.54 *vs* 0.36, P = 0.019).

There were no significant differences in the distributions of the SNP rs8099917 genotypes between the SVR and NR groups. However, the SVR group showed a significantly higher frequency of the T allele (0.74 *vs* NR: 0.57, P = 0.024) but a significantly lower frequency of the G allele (0.26 *vs* 0.43, P = 0.024). Linkage disequilibrium analysis of the SVR group only showed a significant association between rs12979860 and rs8099917 (P = 0.07), similar analysis of the NR group only found no significant associations.

DISCUSSION

Polymorphisms in the genomic sequence related to the *IL28B* gene have been implicated in the natural history of HCV infection and suggested as putative biomarkers for predicting an individual's therapy response[14-17]. In particular, the CC genotype of rs12979860 (*vs* CT) was associated with a two-fold difference in response to HCV treatment, both in Caucasians and African-Americans. Thomas *et al*[18] reported the allele frequencies of rs12979860 in 2371 individuals from 51 populations worldwide, and showed that the C allele occurred at a high frequency in East Asia, an intermediate frequency in Europe and North America, and a relatively low frequency in Africa. In a subsequent study, Fabris *et al*[19] genotyped the *rs12979860* polymorphism in 412 patients with end-stage liver disease due to hepatitis B or C, alcohol abuse, or other causes, and in 344 healthy controls. The patients with viral cirrhosis were found to have a significantly higher frequency of the T allele and of the TT/CT genotypes than the controls; ultimately, the authors theorized that the TT genotype may be associated with severe liver disease while the CC genotype may exert a protective effect. In an Australian-European cohort study of the *rs12979860* polymorphism related to SVR, the TT, TG and GG genotypes were found to be associated[7]. Finally, a Swiss study yielded similar results, with a lower G allele frequency being associated to persistent infection and failure to respond to therapy[6].

Among the few studies that have assessed the role of *IL28B* polymorphisms in a Brazilian population, the SNPs have shown various associations to disease outcome and treatment response. For example, Cavalcante *et al*[20] analyzed rs12979860 in 222 patients infected with admixture HCV genotype 1, 2 and 3 who were treated with standard therapy and found that genotypes CC (rs12979860) and TT (rs8099917) were strongly associated with SVR, while CT/TT (rs12979860) and TG/GG (rs8099917) were associated to treatment failure. In addition, Lunge *et al*[21] analyzed the association between CT (rs12979860) and spontaneous clearance of HCV infection in HIV-co-infected individuals and showed that the CT/TT genotypes conferred nearly 3-fold higher rates for developing CHC, compared to the CC genotype. The current study of *IL28B* polymorphisms' relationship to therapy response, presented herein, confirmed the predictive value of the C allele and CC genotype (rs12979860) that was previously suggested by Cavalcante *et al*[20]. Even though the current study's cohort did not show a statistically significant association of rs8099917 to therapy response, the T allele distribution was related to treatment outcome. This finding may be explained by the lack of HWE in the sample.

Another intriguing finding from the current study is the significant differences in allele and genotype distributions between the healthy controls and HCV-infected patients, which was observed for both SNPs (rs12979860 and rs8099917), with higher C and T alleles as well as CC and TT genotypes in the healthy controls. These data are in accordance with a previous study that demonstrated significant associations for reduced frequencies of the C allele and the CC genotype with chronically-infected hepatitis patients compared to ethnically-matched healthy controls[4]. Together, these findings suggest a strong association between the C allele and higher rates of natural clearance of HCV and support the predicted role of this SNP as a protective factor against HCV infection or persistence.

The full array of biological pathways that are influenced by the *IL28B* gene product and its polymorphisms has yet to be elucidated. Certainly, a gene variant has the ability to affect rates of protein production and it is interesting to consider that Shi *et al*[22] demonstrated that patients with chronic HCV infection have significantly lower mRNA and serum levels of IL28B than either healthy controls or patients who have spontaneously cleared the virus, whereas individuals carrying the rs12979860 CC genotype showed a tendency towards higher levels compared to those carrying the CT or TT genotypes. The SNPs rs12979860 and rs8099917 are located respectively at 3 and 8 kb upstream from the *IL28B* locus, in a region that encodes IFN-λ3, a type 3 interferon with antiviral activity that is mediated through the JAK-STAT pathway upon induction of IFN-stimulated genes. IL28A (IFN-λ2) and IL29 (IFN-λ1) are genomically located adjacent to the *IL28B* gene, and it is possible that the rs12979860 and rs8099917 SNPs may also affect the function of these genes. Indeed, Langhans *et al*[23] showed that IL29 levels were substantially lower in CHC patients and in patients that spontaneously resolved hepatitis, compared to healthy controls; moreover, the levels of *IL29* and *IL28A/B* were shown to be significantly higher in rs12979860 C allele heterozygous patients than in the TT homozygous patients.

The results from the current study of an HCV-infected Brazilian population indicate that the role of *IL28B* SNPs in patients' response to therapy is similar to that of other international populations. Furthermore, the data presented herein on the differential distribution of *rs12979860* and *rs8099917* polymorphisms between HCV-infected patients and a healthy population may contribute to a better understanding of the natural history of this complex disease.

COMMENTS

Background

Most countries continue to rely on drug therapies based on an immunemodulator plus an antiviral (most frequently pegylated-interferon α-2a or -2b and ribavirin), even though this strategy is associated with high failure rates and significant side effects. Therefore, identification of predictive biomarkers of an individual's response to therapy is clinically relevant.

Research frontiers

Since 2009, several genome-wide association studies have yielded data to support the association of two single nucleotide polymorphisms, rs12979860 and rs8099917, located near the interleukin (*IL*)*28B* gene with achievement of sustained virological response to treatment in patients infected with hepatitis C virus (HCV) genotype 1.

Innovations and breakthroughs

This study represents the largest analysis of a Brazilian cohort conducted to date to investigate the potential association of the two most relevant *IL28B* single nucleotide polymorphisms (rs12979860 and rs8099917) in healthy individuals and chronic hepatitis C patients (CHC) treated with the pegylated-interferon α-2a or -2b plus ribavirin combination therapy. The study's findings reveal significant differential distributions of the genotype and allele frequencies for both rs12979860 and rs8099917 between the healthy controls and CHC patients. In addition, the CC genotype of rs12979860 was shown to be associated with the CHC patients' ability to achieve sustained virological response to the standard therapeutic regimens.

Applications

This study's findings support the predicted roles of rs12979860 C allele and rs8099917 T allele as protective factors against HCV infection or persistence and suggest their potential for development as clinical biomarkers of HCV infection and therapeutic management. In general, the results indicate that genotyping *IL28B* polymorphisms in HCV-infected patients is likely to help improve the current standard of care, and may provide an opportunity for clinicians to improve patient outcome by individualizing treatment regimens.

Peer review

The authors investigated the differential distribution of *IL28B* genetic variants between healthy Brazilians in the general population and HCV-infected patients undergoing standard drug therapy based on an immune-modulator plus an antiviral to demonstrate the associations of particular genotypes and alleles with infection and treatment response. This is a well-written and -discussed paper, of particular interest to the Brazil population.

REFERENCES

1 **Tsubota A**, Fujise K, Namiki Y, Tada N. Peginterferon and ribavirin treatment for hepatitis C virus infection. *World J Gastroenterol* 2011; **17**: 419-432 [PMID: 21274371 DOI: 10.3748/wjg.v17.i4.419]

2 **Shirakawa H**, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, Ichijo T, Yoshizawa K, Kiyosawa K, Tanaka E. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* 2008; **48**: 1753-1760 [PMID: 18925643 DOI: 10.1002/hep.22543]

3 **Rehermann B**. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 2009; **119**: 1745-1754 [PMID: 19587449 DOI: 10.1172/JCI39133]

4 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

5 **Tanaka Y**, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-1109 [PMID: 19749757 DOI: 10.1038/ng.449]

6 **Rauch A**, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010; **138**: 1338-145, 1338-145, [PMID: 20060832 DOI: 10.1053/j.gastro.2009.12.056]

7 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]

8 **McCarthy JJ**, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillmann HL, Muir AJ, McHutchison JG. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* 2010; **138**: 2307-2314 [PMID: 20176026 DOI: 10.1053/j.gastro.2010.02.009]

9 **Montes-Cano MA**, García-Lozano JR, Abad-Molina C, Romero-Gómez M, Barroso N, Aguilar-Reina J, Núñez-Roldán A,

González-Escribano MF. Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. *Hepatology* 2010; **52**: 33-37 [PMID: 20578254 DOI: 10.1002/hep.23624]

10 **Te HS**, Jensen DM. Epidemiology of hepatitis B and C viruses: a global overview. *Clin Liver Dis* 2010; **14**: 1-21, vii [PMID: 20123436 DOI: 10.1016/j.cld.2009.11.009]

11 **Osorio RG**. O sistema classificatório de "cor ou raça" do IBGE. *Brasília: Instituto de Pesquisa Econômica Aplicada* 2003; **50**: 1-51

12 **Moreira S**, Garcia RF, Gutberlet A, Bertol BC, Ferreira LE, Pinho Mde S, de França PH. A straightforward genotyping of the relevant IL28B SNPs for the prediction of hepatitis C treatment outcome. *J Virol Methods* 2012; **184**: 93-97 [PMID: 22664181 DOI: 10.1016/j.jviromet.2012.05.024]

13 **Ramos JA**, Ramos AL, Hoffmann L, Perez Rde M, Coelho HS, Urményi TP, Silva R, Rondinelli E, Villela-Nogueira CA. A single nucleotide polymorphism, rs129679860, in the IL28B locus is associated with the viral kinetics and a sustained virological response in a chronic, monoinfected hepatitis C virus genotype-1 Brazilian population treated with pegylated interferon-ribavirin. *Mem Inst Oswaldo Cruz* 2012; **107**: 888-892 [PMID: 23147144]

14 **Elphick M**, Yang JD, Cowen PJ. Effects of carbamazepine on dopamine- and serotonin-mediated neuroendocrine responses. *Arch Gen Psychiatry* 1990; **47**: 135-140 [PMID: 02105705 DOI: 10.1016/j.gastrohep.2010.12.008]

15 **Georgel P**, Schuster C, Zeisel MB, Stoll-Keller F, Berg T, Bahram S, Baumert TF. Virus-host interactions in hepatitis C virus infection: implications for molecular pathogenesis and antiviral strategies. *Trends Mol Med* 2010; **16**: 277-286 [PMID: 20537953 DOI: 10.1016/j.molmed.2010.04.003]

16 **Lindh M**, Lagging M, Arnholm B, Eilard A, Nilsson S, Norkrans G, Söderholm J, Wahlberg T, Wejstål R, Westin J, Hellstrand K. IL28B polymorphisms determine early viral kinetics and treatment outcome in patients receiving peginterferon/ribavirin for chronic hepatitis C genotype 1. *J Viral Hepat* 2011; **18**: e325-e331 [PMID: 21692944 DOI: 10.1111/j.1365-2893.2010.01425.x]

17 **Venegas M**, Villanueva RA, González K, Brahm J. IL28B polymorphisms associated with therapy response in Chilean chronic hepatitis C patients. *World J Gastroenterol* 2011; **17**: 3636-3639 [PMID: 21987611 DOI: 10.3748/wjg.v17.i31.3636]

18 **Thomas DL**, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798-801 [PMID: 19759533 DOI: 10.1038/nature08463]

19 **Fabris C**, Falleti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, Cmet S, Fornasiere E, Fumolo E, Fangazio S, Cerutti A, Minisini R, Pirisi M, Toniutto P. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol* 2011; **54**: 716-722 [PMID: 21146242 DOI: 10.1016/j.jhep.2010.07.019]

20 **Cavalcante LN**, Abe-Sandes K, Angelo AL, Machado TM, Lemaire DC, Mendes CM, Pinho JR, Malta F, Lyra LG, Lyra AC. IL28B polymorphisms are markers of therapy response and are influenced by genetic ancestry in chronic hepatitis C patients from an admixed population. *Liver Int* 2012; **32**: 476-486 [PMID: 22098416 DOI: 10.1111/j.1478-3231.2011.02653.x]

21 **Lunge VR**, da Rocha DB, Béria JU, Tietzmann DC, Stein AT, Simon D. IL28B polymorphism associated with spontaneous clearance of hepatitis C infection in a Southern Brazilian HIV type 1 population. *AIDS Res Hum Retroviruses* 2012; **28**: 215-219 [PMID: 21790472 DOI: 10.1089/aid.2011.0096]

22 **Shi X**, Pan Y, Wang M, Wang D, Li W, Jiang T, Zhang P, Chi X, Jiang Y, Gao Y, Zhong J, Sun B, Xu D, Jiang J, Niu J. IL28B genetic variation is associated with spontaneous clearance of hepatitis C virus, treatment response, serum IL-28B levels in Chinese population. *PLoS One* 2012; **7**: e37054 [PMID: 22649509 DOI: 10.1371/journal.pone.0037054]

23 **Langhans B**, Kupfer B, Braunschweiger I, Arndt S, Schulte W, Nischalke HD, Nattermann J, Oldenburg J, Sauerbruch T, Spengler U. Interferon-lambda serum levels in hepatitis C. *J Hepatol* 2011; **54**: 859-865 [PMID: 21145813 DOI: 10.1016/j.jhep.2010.08.020]

P- Reviewer: Osna NA **S- Editor**: Gou SX **L- Editor**: A
E- Editor: Zhang DN

WJG
World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7405

World J Gastroenterol 2013 November 14; 19(42): 7405-7411
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Short and long-term outcomes of laparoscopic colectomy in obese patients

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Author contributions: Vignali A designed the study and wrote the manuscript; De Nardi P analyzed the data, and revised the article; Ghiradelli L and Di Palo S helped in the acquisition of the data and revised the article; Staudacher C critically revised the manuscript for important intellectual content and gave the final approval.

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Received: August 16, 2013 **Revised:** October 3, 2013
Accepted: October 13, 2013
Published online: November 14, 2013

Abstract

AIM: To investigate the impact of laparoscopic colectomy on short and long-term outcomes in obese patients with colorectal diseases.

METHODS: A total of 98 obese (body mass index > 30 kg/m^2) patients who underwent laparoscopic (LPS) right or left colectomy over a 10 year period were identified from a prospective institutionally approved database and manually matched to obese patients who underwent open colectomy. Controls were selected to match for body mass index, site of primary disease, American Society of Anesthesiologists score, and year of surgery (± 3 year). The parameters analyzed included age, gender, comorbid conditions, American Society of Anaesthesiologists class, diagnosis, procedure, and duration of operation, operative blood loss, and amount of homologous blood transfused. Conversion rate, intra and postoperative complications as were as reoperation rate, 30 d and long-term morbidity rate were also analyzed. For continuous variables, the Student's *t* test was used for normally distributed data the Mann-Whitney *U* test for non-normally distributed data. The Pearson's χ^2 tests, or the Fisher exact test as appropriate, were used for proportions.

RESULTS: Conversion to open surgery was necessary in 13 of 98 patients (13.3%). In the LPS group, operative time was 29 min longer and blood loss was 78 mL lower when compared to open colectomy (P = 0.03, P = 0.0001, respectively). Overall morbidity, anastomotic leak and readmission rate did not significantly differ between the two groups. A trend toward a reduction of wound complications was observed in the LPS when compared to open group (P = 0.09). In the LPS group, an earlier recovery of bowel function (P = 0.001) and a shorter length of stay (P = 0.03) were observed. After a median follow-up of 62 (range 12-132) mo 23 patients in the LPS group and 38 in the open group experienced long-term complications (LPS *vs* open, P = 0.03). Incisional hernia resulted to be the most frequent long-term complication with a significantly higher occurrence in the open group when compared to the laparoscopic one (P = 0.03).

CONCLUSION: Laparoscopic colectomy in obese patients is safe, does not jeopardize postoperative complications and resulted in lower incidence of long-term complications when compared with open cases.

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Key words: Obesity; Colon cancer; Laparoscopy; Right colectomy; Left colectomy; Colorectal disease

Core tip: The best of our knowledge, this is the first case-matched control study reporting long-term complications in obese patients' undergone laparoscopic or open colectomy.

Vignali A, De Nardi P, Ghirardelli L, Di Palo S, Staudacher C. Short and long-term outcomes of laparoscopic colectomy in obese patients. *World J Gastroenterol* 2013; 19(42): 7405-7411

Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7405.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7405

INTRODUCTION

The role of laparoscopic surgery in obese patient with colorectal diseases is still object of debate, according to several papers published in the last decade, laparoscopy is feasible and safe[1-5]. However, controversial results have been reported in term of conversion rate, overall morbidity and reoperation rate, when obese patients are compared to their non-obese counterparts[1-8].

These controversial results could be partially explained based on the relative inexperience of the surgical team possibly due to an on-going learning curve, on the small sample size of the various studies, on the specific definition of obesity and on surgical heterogeneity[3-5,9]. In order to clarify this issue while avoiding some of the aforementioned biases, we have conducted a case-matched study comparing short and long-term outcomes of standardized right or left colectomy performed by laparoscopy or by open surgery in patients with a body mass index (BMI) > 30 kg/m^2. Data were extracted from a prospective collected database.

Main goal of the present study is to assess if the documented benefits of minimally invasive approach could be translated even in a high-risk patients such obese patients.

MATERIALS AND METHODS

Adult obese patients of both sexes candidate to a standardized right or left colectomy and who were consecutively admitted to our department from January 2002 to January 2012 were considered. Patients were identified from a prospective institutionally approved database currently recording demographics, biochemistry values, nutritional status, operative variables, and co-morbidity factors on admission, postoperative outcome and histopathologic findings of patients undergoing colon resection. The case group was selected from 118 obese patients who underwent laparoscopic colectomy identified from the Institutional Colon database. Patients were excluded if they underwent an emergency procedure (n = 13) or non-standardized colon operations (n = 7). Overall, the case group consisted of 98 patients. The control patients were selected from the same institutional database from January 2011, backward until we had identified one control subject for each case. Each case was manually matched with a control that had undergone open resection. The same exclusion criteria used for laparoscopic (LPS) patients were applied to the control patients' selection. The identification of control patients was done by a statistician (GR) who was unaware of postoperative outcomes. Control patients were selected to match for site of primary disease, American Society of Anesthesiologists (ASA) score, and year of surgery (± 3 year). Data collected from the hospital database, medical records and telephone calls were analyzed retrospectively.

Preoperative assessment included clinical examination, serologic evaluation, total colonoscopy and body CT scan. A virtual CT colonoscopy was additionally performed in case of incomplete colonoscopy examination. Co-morbidities on admission, as well as operative risk assessed by ASA score, were analyzed in both groups. The following details of the surgical procedure were recorded in all patients: duration of operation (min), operative blood loss (mL), and amount of homologous blood transfused (mL). Three surgeons (Staudacher C, Di Palo S, Vignali A) with extensive experience in open and laparoscopic colorectal surgery performed all the interventions[10,11]. For all the surgeons, the learning curve for laparoscopic colorectal surgery was completed before starting the present trial[12]. Patients underwent laparoscopic or open resection depending on surgeon's or patient's preference.

A mechanical anastomosis was intracorporeally fashioned in case of left colectomy, while in right colectomy, the specimen was divided extra corporeally (in laparoscopic operations) and an isoperistaltic side-to-side anastomosis was manually fashioned. Conversion to open surgery was defined when an abdominal incision longer than 7 cm was performed or when an abdominal incision was made earlier or differently from what planned at the beginning of the intervention.

All patients were treated according to the same intra and postoperative protocol: epidural analgesia maintenance for 3 d, nasogastric tube removal at the end of surgery, and bladder catheter removal on postoperative 2 d. Clear liquid diet was started on postoperative 1 d as tolerated by the patient. Postoperative infusion of fluids and electrolytes was given to all patients according to clinical requirements.

Tumor classification was done according to the 7th TNM edition[13]. Microbiological analysis and positive cultures proved all infectious complications. Patients were discharged after meeting the following conditions: bowel movement and full recovery of both ambulation ability and oral feeding. Registration of complications and need for an unexpected re-admission were recorded for the first 30 d following operation. The follow-up protocol consisted in outpatient clinic visits at 3 mo intervals for the first 2 years, then at 6 mo intervals for the next 3 years, then once a year. In patients with more than five years follow-up or with a benign disease, a systematic review of chart, office records as well as patient interview were done.

Statistical analysis

Descriptive data were reported as mean, median, standard deviation (SD), number of patients and percentage. For continuous variables, differences between groups were tested with the Student's t test for normally distributed data (based on Kolmogorov-Smirnov test) or the Mann-

Whitney U test for non-normally distributed data. The Pearson's χ^2 test, or the Fisher exact test as appropriate, was used for proportions. A two-sided significance level less than 0.05 were used to indicate statistical significance. Confidence intervals (95%CI) were reported when appropriate. The SPSS™ software package, version 18. For Windows (SPSS Inc, Chicago, IL) was used for all the statistical analyses.

RESULTS

Study population

Ninety-eight obese patients with colorectal diseases, identified from a prospectively maintained database, who underwent laparoscopic right or left colectomy over a 10 year period, were matched with 98 obese patients who underwent open resection.

The two groups were adequately matched for BMI, type of surgery, and operative risk as assessed by their ASA classification (Table 1). Moreover, a similar incidence of co-morbidities on admission was observed between the two groups (Table 2). The mean ± SD (range) BMI (kg/m^2) was 31.9 ± 1.6 (range 30-35.5) in the LPS group, and 32.3 ± 2.1 (range 30.1-36.4) in the open colectomy group. No significant differences were observed between the two groups with respect to demographics, indication for surgery (benign *vs* cancer), tumor stage (among cancer patients) as well as for the incidence of previous operations (Table 1).

Technical feasibility

Conversion to open surgery was necessary in 13 of 98 patients (13.3%). Reasons for conversion were as follows: obesity-hindering vision (n = 5), large tumour infiltrating adjacent organ/s (n = 2), bleeding from the ileocolic pedicle (n = 2), dense adhesions (n = 2), urgent splenectomy (n = 1) and ureteral damage (n = 1). Converted patients remained in the LPS group for an intention to treat analysis. In the converted patients, mean ± SD (range) time from beginning of the conversion procedure to open surgery was 22.5 ± 38.1 (15-80) min.

Table 2 reports the surgical characteristics of the two groups. The operation time was averagely 29 min (95%CI: 24.3-49.3) longer in LPS than open group (P = 0.03). Operative blood loss was 78 mL lower in the LPS group when compared to open surgery (P = 0.0001; 95%CI: 47.1-108.9). No significant differences were observed with respect to perioperative blood transfusion rate between the two groups. Moreover, among cancer patients a similar mean ± SD number of lymph nodes was retrieved in the operative specimen in the two groups (16.6 ± 10.1 in the open group *vs* 17.7 ± 11.5 in the LPS group, P = 0.57).

Table 1 Demographics and clinical characteristics of the two groups *n* (%)

Variable	LPS (n = 98)	Open (n = 98)	P value
Age (yr)	66.9 ± 12.2	68.7 ± 15	0.31
Male/female	52/46	47/51	0.34
ASA score	2.3 ± 0.7	2.4 ± 0.9	0.57
BMI (kg/m^2)	31.9 ± 2.1	32.3 ± 2.5	0.13
Cancer/benign/disease	85/13	79/19	0.34
Previous surgery (%)[1]	8 (8.1)	10 (10.2)	0.30
Type of operation			
Right colectomy	57	57	
Left colectomy	41	41	
Tumour stage	n = 85	n = 79	0.77
Stage 0	13 (15.2)	9 (11.3)	
Stage 1	14 (16.5)	13 (16.3)	
Stage 2	29 (34.2)	32 (40.6)	
Stage 3	25 (29.4)	20 (25.4)	
Stage 4	4 (4.7)	5 (6.4)	

[1]Only abdominal surgeries were included; Values are reported as mean ± SD or n (%). BMI: Body mass index; LPS: Laparoscopic; ASA: American Society of Anesthesiologists.

Postoperative outcomes

In the LPS group, a death occurred as a sequelae of an anastomotic leakage on postoperative day 5. No significant differences between the two groups were observed with respect to overall morbidity rate as well as for technical complications such as anastomotic leakage. Moreover, a similar incidence of hospital re-admission within 30 d and of reoperation rate was observed in the two groups (Table 2). Conversely, in the open group, the occurrence of wound complications was more than double when compared to the LPS group and the difference showed a trend toward a statistical significance (P = 0.09). In both groups, more than 30% of the postoperative infections occurred after discharge. A similar incidence of respiratory and cardiac complications was observed in the two groups (Table 3). When the converted cases were compared to the laparoscopically completed ones, a trend toward a longer duration of surgery (P = 0.07) and a longer length of stay was observed (P = 0.002). Converted patients experienced a higher complications rate when compared to patients who completed the operation laparoscopically; however the difference failed to reach statistical significance (22.8 *vs* 32.4, P = 0.62). Similar results in term of length of stay and overall complication rate were observed when converted and open surgery patients were compared (Table 4). Mean ± SD recovery of oral food intake occurred after 2.1 (1) d in the LPS and after 3.5 ± 1.5 d in the open surgery group (P = 0.001). Mean (median, SD) length of stay was 8.6 (8; 3.1) d in the LPS group and 10.4 (10; 4.9) d in the open surgery group (P = 0.03).

Long-term outcomes

At a median follow-up of 62 (range 12-132) mo, 23 patients in the LPS group and 38 in the open surgery group experienced long-term complications (LPS *vs* open, P = 0.03; Table 2). Incisional hernia was the most common complication in both groups and and its occurrence was more frequent in the open surgery group when compared to the laparoscopic one (P = 0.03). Among the converted patients, a 30.8% (4/13 pts) incisional hernia rate was observed.

Table 2 Comparison of co-morbidities, variables, complications (30 d morbidity) and Long-term complications between the two groups in study n (%)

		LPS (n = 98)	Open (n = 98)	P value
Co-morbidities	Diabetes	14	11	0.66
	Coronary artery disease[1]	13	15	0.84
	Hypertension	43	52	0.25
	Smoker	10	14	0.51
	Chronic obstructive pulmonary disease	5	3	0.72
	Steroid use	2	3	0.91
Variable	Operative time (min)	193 (71)	164 (111)	0.03
	Blood loss (mL)	177 (76)	255 (102)	0.0001
	No of transfused patients	11 (11.2)	16 (16.3)	0.33
	Conversion rate	13 (13.3)	NA	
Complications (30 d morbidity)	Overall	27 (27.6)	33 (33.7)	0.4
	Infectious[2]	13 (13.7)	21 (21.7)	0.13
	Noninfectious[2]	9 (9.2)	10 (10.2)	0.8
	Anastomotic leak[2]	7 (7.1)	5 (5.1)	0.78
	Readmission	7 (7.1)	11 (11.2)	0.56
	Reoperation	8 (8.1)	9 (9.2)	0.8
	Length of stay	8 (3.1)	10.4 (4.9)	0.03
	Mortality	1 (1.02)	0 (0.0)	0.9
Long-term complications	Incisional hernia	17	31	0.03
	Intestinal obstruction	5	6	0.48
	Anastomotic stricture	4	4	0.86
	Overall	23	38	0.03

[1]Including: History of angina, percutaneous cardiac intervention, cardiac operation, or myocardial infarction within 6 mo of operation; [2]Number of single type of complication do not add up to the number of overall complication within the two groups because of the possible occurrence of more than one type of complication in some patients; all values within parenthesis indicate percentage values. LPS: Laparoscopic.

Table 3 Postoperative complications in details n (%)

	LPS (n = 98)	Open (n = 98)
Infectious complications		
Wound complications[1]		
Wound infections	8 (8.2)	15 (15.4)
Wound disruption	-	2 (2.04)
Abdominal abscess	1 (1.06)	2 (2.04)
Pneumonia	2 (2.04)	3 (3.1)
Urinary tract	2 (2.04)	1 (1.06)
Non-infectious complications		
Cardiologic	2 (2.04)	3 (3.1)
Ileus	3 (3.1)	3 (3.1)
Intestinal obstruction	3 (3.1)	4 (4.1)
Bleeding	1 (1.01)	-

[1]Laparoscopic (LPS) *vs* open, P = 0.09.

DISCUSSION

The global prevalence of obesity means that surgeons are increasingly faced with these high-risk patients. The choice of optimal operative approach and technique becomes extremely actual and crucial. The vast majority of the studies available in the literature addressing the issue of mini-invasive approach and obesity compares obese patients to their non-obese counterparts[2-6]. In our opinion, in order to better evaluate the effective impact of mini-invasive approach in obese patients, it is important to compare open and laparoscopic colectomy outcomes in the specific population of obese patients only. A definitive answer whether laparoscopic surgery would be preferable in the obese to the open approach can only be obtained by a randomized controlled trial. To the best of our knowledge, there are no RCTs in the literature specifically addressing this issue. To limit the biases related to the design of the study and in an attempt to minimize surgical heterogeneicity[14], a single center case-matched study was performed including only well standardized surgical procedures.

In previous studies, obesity has been identified as one of the factors associated with a higher conversion rate[15-19]. In the present trial, a conversion rate of 13.3% has been obtained, which is within the range (0%-39%) previously reported by other studies dealing with this issue[1-6]. This rate is however higher when compared to the 2.6% conversion rate after laparoscopic right colectomy or the 5.2% conversion rate in left LPS colectomies reported in studies performed by our Institution in the non-obese population[20,21]. These findings are in accordance with results from Tekkis and co-workers who identified obesity as an independent predictor of conversion to open surgery at multivariate analysis with an odd-ratio of 2.2 for patients with a BMI > 28.5 kg/m^2 derived from a large series 1253 subjects[22]. Moreover an increasing BMI was associated with a proportionally higher conversion rates in data extracted from the laparoscopic colorectal surgery study group (LCSSG)” on 5853 recruited patients[23].

A possible argument against the adoption of the mini-invasive approach in obese patients is that converted patients resulted in poor short and long-term outcomes when compared to patients who successfully completed the operation laparoscopically[24,25]. In our experience,

Table 4 Outcomes of laparoscopically completed, converted and open cases

Variable	LPS completed (n = 85)	Converted (n = 13)	Open (n = 98)	P value
Operative time (min)	175 (61)	210 (86)	164 (111)	0.07^1 0.08^2
Morbidity rate	22.8%	32.4%	33.7%	0.55^1 0.91^2
Length of stay (d)	7.2 (2.5)	9.6 (3.2)	10.4 (4.9)	0.002^1 0.52^2

^{1}Laparoscopic (LPS) completed *vs* converted; ^{2}Converted *vs* open. Values are expressed as mean (SD) or percentages.

in the converted patients, no significant difference was observed in term of postoperative morbidity when compared to laparoscopically completed cases. Similar results were reported by other authors[26,27]. Possible explanation may include the wide experience of the surgical team, in the appropriate patients' selection, or to the rapid decision to convert thus minimizing potentially adverse outcomes[1,4,10,22].

In the present series, the overall morbidity rates did not differ between the two groups. Similarly, despite the incidence of infectious complications was about twice higher in patients in the open surgery group when compared to laparoscopic (23.6% *vs* 11%), the difference failed to reach statistical significance.

Only with respect to wound complications, a trend toward a lower rate was observed in the LPS group. These latter findings are consistent with the data recently reported by Wick *et al*[28] and Mustain *et al*[29], who identified open surgery as an independent risk factors for surgical site infections in a large series of obese patients undergoing laparoscopic and open colorectal surgery[28,29].

In the present series, the incidence of anastomotic leakage was similar in the two groups. Delaney and coworkers reported similar results in the only other, to the best of our knowledge, case-matched study comparing laparoscopic to open colectomy in obese patients,. These authors reported an absence of statistical difference, both for the overall complications as well as for anastomotic leakage rate[27]. The absence of an adverse impact of laparoscopic colectomy on anastomotic leakage rate in the obese patients undergone laparoscopic colectomy has been recently supported by a review paper by Martin and Stocchi[9] reporting data from high volume institutions.

Moreover, patients in the LPS group experienced a shorter recovery of bowel function and a shorter length of stay when compared to their open surgery counterparts. These findings are consistent with the results reported by a large meta-analyses analyzing the outcomes of 2512 procedures from 12 RCT trials comparing LPS and the open approach for colorectal diseases in the non-obese population[30]. In our series, the earlier recovery of bowel function, the shorter length of stay as well as the lower intraoperative blood loss and a trend toward a reduction of wound complications observed in the LPS group, deserve major consideration, as these findings indicate that some of the wide-reported short-term benefits of the mini-invasive approach are maintained even in high-risk patients, also suggesting that paradoxically, these are the patients who stands to benefit the most laparoscopic surgery.

With respect to long-term complications, patients in the LPS group experienced a significantly lower incidence of overall complications when compared to their open surgery counterparts. In particular a higher incisional hernia rate was observed in the open surgery group. Few data are available in the literature on this subject reporting controversial results. No difference in term of incisional hernias between laparoscopic or open approach has been reported or, conversely the mini-invasive approach was preferred when obese *vs* non obese patients are compared[31,32]. To the best of our knowledge, no study has reported data on long-term complications in obese patients treated with mini-invasive or with conventional approach. The mechanisms for incisional hernia occurrence have not been yet clarified. Potential risk factors have been identified and categorized into patient-related (advanced age, obesity, nutritional status,) disease and surgery-related such as emergency operation, postoperative wound infections, reoperations and others[33-35]. Moreover, an association was recently reported by Rullier *et al*[36] between the rate of hernia and the length of incision. These authors found an incisional hernia rate of 33.0% at median follow-up period of 51 mo in open group compared to 13.5% in the LPS group of patients who underwent rectal resection.

In conclusions, although there are some limitations to the study resulting from the non-randomized design, to the possible bias in patient selection for laparoscopic surgery, and to the fact that patients were treated in a single institution, our findings indicate that laparoscopic surgery can be safely performed in obese patients with colorectal disease. Moreover, minimally invasive approach has no adverse impact on postoperative complications, resulting in reduced length of stay and lower incidence of long-term incisional hernia when compared to conventional colectomy.

ACKNOWLEDGMENTS

We thank Giovanni Radaelli MD, PHD for performing the statistical analysis of the data.

COMMENTS

Background

The safety and the benefits of laparoscopic colon resection, including less post-operative pain, faster recovery of bowel function, earlier mobilization, less morbidity, reduction of hospital stay, and smaller scars, have been underlined by several studies, making it now the preferred approach in the surgical management of many colorectal diseases. Obese patients are considered to be at high perioperative risks. Therefore laparoscopic surgery may be particularly advantageous in obese patients. On the other hand a colorectal resection is more difficult in obese subjects due to obesity hindering visualization and dissection of tissue planes that lead to longer operative time and increase blood transfusion requirement, thus possibly impair the benefits of laparoscopic colectomy.

Research frontiers

The study performed a single center study in which patients were matched for body mass index, site of primary disease, American Society of Anesthesiologists score, and year of surgery. Moreover only well standardized surgical procedures (left and right colectomy) were included. However, in order to better evaluate the real impact of minimally-invasive approach in obese patients, a randomized controlled trial should be conducted.

Innovations and breakthroughs

The vast majority of previous studies, focusing on laparoscopic colectomies in obese patients, compared obese to non-obese subjects. No randomized study has been conducted. Only one case matched study compared open and laparoscopic approach in obese patients. This is the first study that analyses long-term outcomes. The principals finding of this study is that the documented benefits of laparoscopic colon resection could be translated even in a high-risk group, such obese patients, since this approach did not impair postoperative outcomes and resulted in lower incidence of long-term complications when matched with open surgery cases.

Applications

Providing that experienced surgeons are involved, laparoscopic colorectal resection should be offered to obese patients considering its benefits when compared to conventional open surgery.

Peer review

The authors analyze the feasibility of laparoscopic colectomy in obese patients. There are not randomized trials comparing the outcome of colectomy in obese and non-obese patients. The results confirm previous studies on the efficacy and safety of laparoscopic left and right colectomy in obese subjects. The literature on this topic is scarce, and, although another case-match study was previously published, this is the only one that analyses long term results.

REFERENCES

1 **Dindo D**, Muller MK, Weber M, Clavien PA. Obesity in general elective surgery. *Lancet* 2003; **361**: 2032-2035 [PMID: 12814714 DOI: 10.1016/S0140-6736(03)13640-9]

2 **Leroy J**, Ananian P, Rubino F, Claudon B, Mutter D, Marescaux J. The impact of obesity on technical feasibility and postoperative outcomes of laparoscopic left colectomy. *Ann Surg* 2005; **241**: 69-76 [PMID: 15621993 DOI: 10.1097/01.sla.0000150168.59592.b9]

3 **Gendall KA**, Raniga S, Kennedy R, Frizelle FA. The impact of obesity on outcome after major colorectal surgery. *Dis Colon Rectum* 2007; **50**: 2223-2237 [PMID: 17899278 DOI: 10.1007/s10350-007-9051-0]

4 **Pikarsky AJ**, Saida Y, Yamaguchi T, Martinez S, Chen W, Weiss EG, Nogueras JJ, Wexner SD. Is obesity a high-risk factor for laparoscopic colorectal surgery? *Surg Endosc* 2002; **16**: 855-858 [PMID: 11997837 DOI: 10.1007/s004640080069]

5 **Makino T**, Shukla PJ, Rubino F, Milsom JW. The impact of obesity on perioperative outcomes after laparoscopic colorectal resection. *Ann Surg* 2012; **255**: 228-236 [PMID: 22190113 DOI: 10.1097/SLA.0b013e31823dcbf7]

6 **Benoist S**, Panis Y, Alves A, Valleur P. Impact of obesity on surgical outcomes after colorectal resection. *Am J Surg* 2000; **179**: 275-281 [PMID: 10875985 DOI: 10.1016/S0002-9610(00)00337-8]

7 **Kamoun S**, Alves A, Bretagnol F, Lefevre JH, Valleur P, Panis Y. Outcomes of laparoscopic colorectal surgery in obese and nonobese patients: a case-matched study of 180 patients. *Am J Surg* 2009; **198**: 450-455 [PMID: 19285301 DOI: 10.1016/j.amjsurg.2008.09.022]

8 **Schwandner O**, Farke S, Schiedeck TH, Bruch HP. Laparoscopic colorectal surgery in obese and nonobese patients: do differences in body mass indices lead to different outcomes? *Surg Endosc* 2004; **18**: 1452-1456 [PMID: 15791368 DOI: 10.1007/s00464-003-9259-6]

9 **Martin ST**, Stocchi L. Laparoscopic colorectal resection in the obese patient. *Clin Colon Rectal Surg* 2011; **24**: 263-273 [PMID: 23204942 DOI: 10.1055/s-0031-1295690]

10 **Vignali A**, Di Palo S, Tamburini A, Radaelli G, Orsenigo E, Staudacher C. Laparoscopic vs. open colectomies in octogenarians: a case-matched control study. *Dis Colon Rectum* 2005; **48**: 2070-2075 [PMID: 16086219 DOI: 10.1007/s10350-005-0147-0]

11 **Vignali A**, Braga M, Zuliani W, Frasson M, Radaelli G, Di Carlo V. Laparoscopic colorectal surgery modifies risk factors for postoperative morbidity. *Dis Colon Rectum* 2004; **47**: 1686-1693 [PMID: 15540300 DOI: 10.1007/s10350-004-0653-5]

12 **Braga M**, Vignali A, Zuliani W, Radaelli G, Gianotti L, Toussoun G, Carlo V. Training period in laparoscopic colorectal surgery. *Surg Endosc* 2002; **16**: 31-35 [PMID: 11961600]

13 **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474 [PMID: 20180029 DOI: 10.1245/s10434-010-0985-4]

14 **Senagore AJ**, Delaney CP, Brady KM, Fazio VW. Standardized approach to laparoscopic right colectomy: outcomes in 70 consecutive cases. *J Am Coll Surg* 2004; **199**: 675-679 [PMID: 15501105 DOI: 10.1016/j.jamcollsurg.2004.06.021]

15 **Senagore AJ**, Delaney CP, Madboulay K, Brady KM, Fazio VW. Laparoscopic colectomy in obese and nonobese patients. *J Gastrointest Surg* 2003; **7**: 558-561 [PMID: 12763416 DOI: 10.1016/S1091-255X(02)00124-5]

16 **Denost Q**, Quintane L, Buscail E, Martenot M, Laurent C, Rullier E. Short- and long-term impact of body mass index on laparoscopic rectal cancer surgery. *Colorectal Dis* 2013; **15**: 463-469 [PMID: 23534683 DOI: 10.1111/codi.12026]

17 **Bège T**, Lelong B, Francon D, Turrini O, Guiramand J, Delpero JR. Impact of obesity on short-term results of laparoscopic rectal cancer resection. *Surg Endosc* 2009; **23**: 1460-1464 [PMID: 19116737 DOI: 10.1007/s00464-008-0266-5]

18 **Cima RR**, Hassan I, Poola VP, Larson DW, Dozois EJ, Larson DR, O'Byrne MM, Huebner M. Failure of institutionally derived predictive models of conversion in laparoscopic colorectal surgery to predict conversion outcomes in an independent data set of 998 laparoscopic colorectal procedures. *Ann Surg* 2010; **251**: 652-658 [PMID: 20195150 DOI: 10.1097/SLA.0b013e3181d355f7]

19 **Park JW**, Lim SW, Choi HS, Jeong SY, Oh JH, Lim SB. The impact of obesity on outcomes of laparoscopic surgery for colorectal cancer in Asians. *Surg Endosc* 2010; **24**: 1679-1685 [PMID: 20039065 DOI: 10.1007/s00464-009-0829-0]

20 **Braga M**, Frasson M, Vignali A, Zuliani W, Di Carlo V. Open right colectomy is still effective compared to laparoscopy: results of a randomized trial. *Ann Surg* 2007; **246**: 1010-104; discussion 1010-104; [PMID: 18043103 DOI: 10.1097/SLA.0b013e31815c4065]

21 **Braga M**, Frasson M, Zuliani W, Vignali A, Pecorelli N, Di Carlo V. Randomized clinical trial of laparoscopic versus open left colonic resection. *Br J Surg* 2010; **97**: 1180-1186 [PMID: 20602506 DOI: 10.1002/bjs.7094]

22 **Tekkis PP**, Senagore AJ, Delaney CP. Conversion rates in laparoscopic colorectal surgery: a predictive model with, 1253 patients. *Surg Endosc* 2005; **19**: 47-54 [PMID: 15549630 DOI: 10.1007/s00464-004-8904-z]

23 **Scheidbach H**, Benedix F, Hügel O, Kose D, Köckerling F, Lippert H. Laparoscopic approach to colorectal procedures in the obese patient: risk factor or benefit? *Obes Surg* 2008; **18**: 66-70 [PMID: 18080169 DOI: 10.1007/s11695-007-9266-0]

24 **Scheidbach H**, Garlipp B, Oberländer H, Adolf D, Köckerling F, Lippert H. Conversion in laparoscopic colorectal cancer surgery: impact on short- and long-term outcome. *J Laparoendosc Adv Surg Tech A* 2011; **21**: 923-927 [PMID: 22011276 DOI: 10.1089/lap.2011.0298]

25 **Marusch F**, Gastinger I, Schneider C, Scheidbach H, Konradt J, Bruch HP, Köhler L, Bärlehner E, Köckerling F. Importance of conversion for results obtained with laparoscopic colorectal surgery. *Dis Colon Rectum* 2001; **44**: 207-214; discussion 214-216 [PMID: 11227937 DOI: 10.1007/bf02234294]

26 **Khoury W**, Stocchi L, Geisler D. Outcomes after laparoscopic intestinal resection in obese versus non-obese patients. *Br J Surg* 2011; **98**: 293-298 [PMID: 21110332 DOI: 10.1002/bjs.7313]

27 **Delaney CP**, Pokala N, Senagore AJ, Casillas S, Kiran RP, Brady KM, Fazio VW. Is laparoscopic colectomy applicable to patients with body mass index & gt; 30? A case-matched comparative study with open colectomy. *Dis Colon Rectum* 2005; **48**: 975-981 [PMID: 15793638 DOI: 10.1007/s10350-004-0941-0]

28 **Wick EC**, Hirose K, Shore AD, Clark JM, Gearhart SL, Efron J, Makary MA. Surgical site infections and cost in obese patients undergoing colorectal surgery. *Arch Surg* 2011; **146**: 1068-1072 [PMID: 21576597 DOI: 10.1001/archsurg.2011.117]

29 **Mustain WC**, Davenport DL, Hourigan JS, Vargas HD. Obesity and laparoscopic colectomy: outcomes from the ACS-NSQIP database. *Dis Colon Rectum* 2012; **55**: 429-435 [PMID: 22426267 DOI: 10.1097/DCR.0b013e31823dfb17]

30 **Abraham NS**, Young JM, Solomon MJ. Meta-analysis of short-term outcomes after laparoscopic resection for colorectal cancer. *Br J Surg* 2004; **91**: 1111-1124 [PMID: 15449261 DOI: 10.1002/bjs.4640]

31 **Sonoda T**, Pandey S, Trencheva K, Lee S, Milsom J. Longterm complications of hand-assisted versus laparoscopic colectomy. *J Am Coll Surg* 2009; **208**: 62-66 [PMID: 19228504 DOI: 10.1016/j.jamcollsurg.2008.09.003]

32 **Balentine CJ**, Marshall C, Robinson C, Wilks J, Anaya D, Albo D, Berger DH. Obese patients benefit from minimally invasive colorectal cancer surgery. *J Surg Res* 2010; **163**: 29-34 [PMID: 20538294 DOI: 10.1016/j.jss.2010.03.063]

33 **Song IH**, Ha HK, Choi SG, Jeon BG, Kim MJ, Park KJ. Analysis of risk factors for the development of incisional and parastomal hernias in patients after colorectal surgery. *J Korean Soc Coloproctol* 2012; **28**: 299-303 [PMID: 23346508 DOI: 10.3393/jksc.2012.28.6.299]

34 **Murray BW**, Cipher DJ, Pham T, Anthony T. The impact of surgical site infection on the development of incisional hernia and small bowel obstruction in colorectal surgery. *Am J Surg* 2011; **202**: 558-560 [PMID: 21924402 DOI: 10.1016/j.amjsurg]

35 **Samia H**, Lawrence J, Nobel T, Stein S, Champagne BJ, Delaney CP. Extraction site location and incisional hernias after laparoscopic colorectal surgery: should we be avoiding the midline? *Am J Surg* 2013; **205**: 264-267; discussion 268 [PMID: 23375702 DOI: 10.1016/j.amjsurg]

36 **Laurent C**, Leblanc F, Bretagnol F, Capdepont M, Rullier E. Long-term wound advantages of the laparoscopic approach in rectal cancer. *Br J Surg* 2008; **95**: 903-908 [PMID: 18551506 DOI: 10.1002/bjs.6134]

P- Reviewers: Kanda T, Orsenigo E, Venskutonis D
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Zhang DN

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7412

World J Gastroenterol 2013 November 14; 19(42): 7412-7418
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

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BRIEF ARTICLE

Is increased red cell distribution width an indicating marker of nonalcoholic steatohepatitis and fibrotic stage?

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Received: June 9, 2013 **Revised:** August 19, 2013
Accepted: September 15, 2013
Published online: November 14, 2013

Abstract

AIM: To evaluate the red cell distribution width (RDW) as an indicator of the presence of non-alcoholic steatohepatitis (NASH) and its association with fibrotic scores.

METHODS: A retrospective study was carried out that included sixty-two biopsy proven NASH, 32 simple steatosis patients and 30 healthy controls. The correlation between the clinical and histopathological features of NASH patients and RDW values was evaluated. Liver fibrosis scores were measured using a 0 to 4 point scale and were divided in to two groups; fibrosis scores 0-1 were termed mild and fibrosis scores 2-4 were termed advanced fibrosis. RDW values were compared between NASH, simple steatosis and healthy controls. Univariate and multivariate analyses were performed to evaluate the independent predicting factors for the presence of liver fibrosis caused by NASH.

RESULTS: Patients with NASH had higher RDW values compared with simple steatosis and healthy control groups [14.28% ± 0.25% *vs* 13.37% ± 0.12%, 12.96% ± 0.14% (P < 0.01), respectively]. Patients with advanced fibrosis had higher RDW values than the mild fibrosis group (15.86% ± 0.4% *vs* 13.63% ± 0.67%, P < 0.01, respectively). RDW also correlated with fibrotic scores (r = 0.579 and P < 0.01). The variables that were significant in the univariate analysis were evaluated in multivariate logistic regression analysis, and RDW was an independent predicting factor of NASH (OR = 1.75, 95%CI: 1.129-2.711, P < 0.05).

CONCLUSION: RDW a new non-invasive marker that can be used to demonstrate the presence of NASH and indicate advanced fibrotic scores.

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Key words: Non-alcoholic steatohepatitis; Liver fibrosis; Red cell distribution width; Simple steatosis; Non-invasive marker; Liver biopsy

Core tip: We evaluated the role of red cell distribution width (RDW) as an indication of nonalcoholic steatohepatitis by comparing the values of biopsy proven non-alcoholic steatohepatitis (NASH) patients with simple steatosis and healthy controls. Independent predictors of the presence of NASH and advanced liver fibrosis were evaluated by using multivariate logistic regression analyses and RDW was a statistically significant independent predictor.

Cengiz M, Candır BA, Yılmaz G, Akyol G, Ozenirler S. Is increased red cell distribution width an indicating marker of nonalcoholic steatohepatitis and fibrotic stage? *World J Gastroenterol* 2013; 19(42): 7412-7418 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7412.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7412

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is part of the spectrum of non-alcoholic fatty liver disease (NAFLD), which comprises simple fatty liver, NASH, associated advanced fibrosis and cirrhosis[1]. NAFLD has become one of the most common forms of chronic liver diseases in the United States and worldwide, with a prevalence of 10%-24%[2-5]. NASH is characterized by ballooning, degeneration, and lobular inflammation with various stages of fibrosis. Lobular inflammation is a hallmark of NASH, which is characterized by infiltration of lymphocytes, mononuclear cells and polymorphonuclear neutrophils. Steatosis is present in all cases and affects the hepatic lobules[6]. Simple steatosis is a benign condition with minimum progression, despite a high rate of progression to cirrhosis in 15%-25% of patients with NASH[7]. Liver fibrosis is a result of chronic injury[8]. In the absence of approved treatment modalities for NASH, care should be taken on the detection of advanced fibrosis. A liver biopsy is the only method for distinguishing NASH from other diagnoses and permits the evaluation of the damage and fibrosis of the liver. However a biopsy is an invasive and expensive procedure with important complications, such as bleeding and perforation. Therefore, there has been a search for noninvasive methods of detecting the presence of NASH and liver fibrosis to avoid these procedure-related complications.

Chronic inflammation plays a significant role in disease progression to NASH[9]. In addition, some studies have shown that certain inflammatory cytokines were higher in patients with NASH[10,11].

Red cell distribution width (RDW) is a parameter of the variation of circulating red cells. This parameter demonstrates the heterogeneity of red cell volume and is a component of the complete blood count (CBC). The RDW is also a widely available, inexpensive and easily repeatable marker that measures red blood cell (RBC) volume variability. Recent reports have demonstrated that elevated RDW values were related to negative outcomes in cardiovascular and metabolic disorders, colon cancer and stroke independent of hemoglobin (HGB) values[12-16]. The association between RDW values and the severity of liver diseases has been also reported in two recent studies[17,18].

In this study, we aimed to investigate the clinical utility of RDW for indicating the presence of NASH in comparison with simple steatosis and healthy controls. We also evaluated whether there is an association between RDW values, fibrotic stages and histological features of NASH.

MATERIALS AND METHODS

Patients

This retrospective study was performed in Gazi University Department of Gastroenterology, a tertiary reference center (Ankara, Turkey), between January 2010 and May 2013. All the patients who had persistently elevated liver enzymes and hepatosteatosis on ultrasonography, in the absence of any causes of elevated aminotransferases, were evaluated in the study. Among them, patients who had histopathology consistent with NASH and simple steatosis were included in the study. The control group was created among individuals who had normal aminotransferase levels and normal abdominal ultrasonography. Patients who were diagnosed with viral hepatitis, sclerosing cholangitis, primary biliary cirrhosis, autoimmune hepatitis, hemochromatosis, Wilson's disease, alpha-1-antitrypsin deficiency, malignancy and drug-induced liver disease were excluded. Other exclusion criteria were alcohol consumption of > 20 g/d for men and > 10 g/d for women, presence of infectious diseases on admission, chronic renal diseases, collagen vascular diseases, malignancies, hematological diseases that might impair red cell production (such as iron deficiency, B12 or folate deficiency), increased red cell destruction, hemoglobinopathies, blood transfusions, bone marrow depression, usage of anticoagulant drugs, non-steroid anti-inflammatory drugs and hepatotoxic drugs. Patients who had diabetes mellitus were on oral antidiabetics and/or insulin therapy. Patients who had hypertension were all using angiotensin-converting enzyme inhibitors and were under control in terms of hypertension.

Clinical and laboratory assessment

Demographical, clinical and laboratory data were collected and registered in a database by an uninformed clinician to prevent bias. Body mass index was calculated by dividing weight in kilograms (kg) by the square of height in meter (m) as (kg/m^2).

Before the liver biopsy, venous blood samples were obtained from the antecubital vein from all patients between 8.30 and 10.00 am, after fasting for at least 8-12 h. A CBC containing RDW, hemoglobin (HB), white blood cell (WBC), neutrophils, lymphocytes, platelets and mean platelet volume (MPV) was performed with a Beckman Coulter Gen-S automated analyzer (High Wycombe, United Kingdom) within 2 h after obtaining the blood samples. RDW was calculated by the Beckman Coulter automated analyzer as RDW = SD/MCV × 100%. (The standard deviation of the volume of red blood cell (SD)/[(mean corpuscle volume) (MCV)] × 100%. RDW was calculated by dividing the SD by the MCV and then multiplying that result by 100. The SD represented the volume of erythrocytes or RBCs that were in the blood smear.

A Roche Modular System auto analyzer(Roche COBAS INTEGRA 800 (Indianapolis, United States) determined the Albumin, creatinine, blood urine nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ glutamyl aminotransferase (GGT) and alkaline phosphatase (ALP) levels. All the laboratory analyses were performed in our hospital's hematology laboratory.

Histopathological evaluation of the liver

Percutaneous liver biopsy was performed using a 16-G disposable needle by a skilled clinician. All liver biopsy specimens included 12 or more complete portal tracts

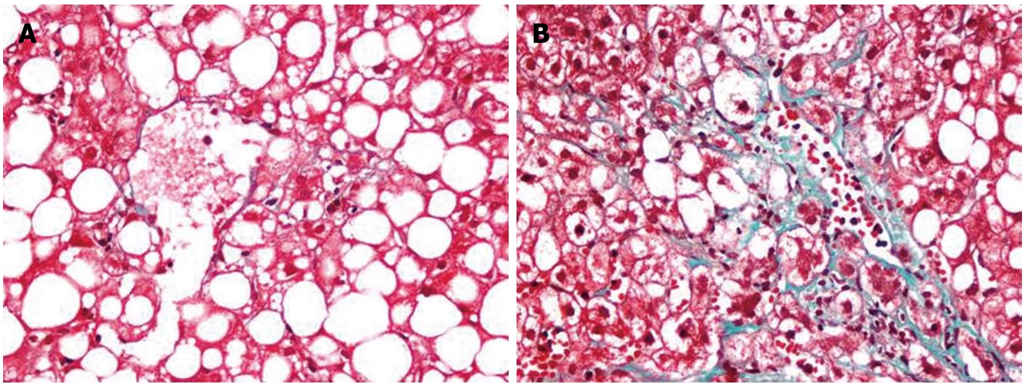

Figure 1 Mild perivenular-perisinusoidal fibrosis (A) and advanced perivenular-perisinusoidal, and periportal fibrosis (B) in non-alcoholic steatohepatitis. A, B: Trichrome stain, x 400.

and were longer than 20-25 mm. All liver tissue samples were evaluated by the same experienced hepatopathologist who was blinded to the patients' clinical and laboratory data. Hematoxylin and eosin (HE) and Masson trichrome stains were used for histopathological diagnoses of formalin-fixed paraffin-embedded liver tissues. The diagnosis of NASH was evaluated on Brunt's Criteria[19]. Histological characteristics were graded according to the NAFLD scoring system recommended by the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network[20]. Steatosis was graded as 0 ⩽ 5%; 1 ⩾ 33%-66%; 3 ⩾ 66% lobular inflammation was graded as 0 = no foci; 1 ⩽ 2 foci; 2 = 2-4 foci; 3 ⩾ 4 foci; and ballooning was graded as 0 = none; 1 = few ballooning cells; 2 = many ballooning cells, respectively.

Depending on the recommendations of Brunt's criteria steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2) were then combined to establish the NAFLD activity score (0-8). Fibrosis was also scored as 0 = no fibrosis; 1 = periportal or perisinusoidal fibrosis; 2 = perisinusoidal and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis**.** While mild fibrosis was defined with fibrotic score ⩽ 1 advanced fibrosis was defined with fibrotic score > 1. Mild fibrosis caused by NASH is shown in Figure 1A and advanced fibrosis in Figure 1B.

Ethics

The study was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review board.

Statistical analysis

Statistical analyses were performed using the SPSS software version 17 and MedCalc version 12. The variables were investigated by using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they were normally distributed. Non-normally distributed and non-parametric variables were compared between groups using the Mann-Whitney *U* test where appropriate. The χ^2 test, where appropriate, was used to compare the propositions in different groups. As the RDW was not normally distributed, the Kruskal-Wallis test was used for comparisons among NASH, simple steatosis and healthy control groups. The Mann-Whitney *U* test was used to test the significance of pairwise differences, using Bonferroni correction to adjust for multiple comparisons. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors of NASH. To assess model fit, we used Hosmer-Lemeshow goodness of fit statistics. Spearman rank correlation coefficients (r) were calculated to assess the correlation between RDW and liver histopathological features (inflammation, NAS score, the degree of steatosis and degree of fibrosis) and clinical characteristics of NASH patients. Receiver operating characteristics (ROC) analysis was used to evaluate the role of RDW in distinguishing subjects with NASH and fibrosis. ROC analysis was also used to distinguish advanced and mild fibrotic stage groups. Cutoff values that maximized both sensitivity and specificity were chosen for the NASH group. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics and liver histology

Sixty-two biopsy proven NASH patients, 32 biopsy proven simple steatosis patients and 30 healthy controls were recruited. The median ages of patients with NASH, simple steatosis and healthy controls were 49.5 years (22-77 years), 48 years (24-71 years) and 48 years (24-72 years), respectively. There was no statistically significant difference between the ages of participants. The mean BMI (kg/m^2) of NASH, simple steatosis and healthy controls were 27.13 ± 0.37, 25.97 ± 0.67 and 24.22 ± 0.45 kg/m^2, respectively ($P < 0.01$). The platelet counts among NASH, simple steatosis and healthy controls were 245.5 ± 34.73, 244.5 ± 13.8 and 260 ± 4.47 × 10^3/mL, respectively ($P > 0.05$). However, there was an inversely significant correlation between platelet counts and fibrotic scores (r = -0.335 and $P < 0.01$). Among the NASH group 30/62 (48.3%), steatosis group 18/32 (56.2%) and

Table 1 Demographic and laboratory features of non-alcoholic steatohepatitis, simple steatosis and healthy control groups

Factor	NASH group (*n* = 62)	Steatosis group (*n* = 32)	Healthy control group (*n* = 30)	*P*-value
Age mean ± SD	48.81 ± 12.21	47.25 ± 12.58	48.03 ± 9.54	NS
Gender (male)	30 (48.3)	18 (56.2)	18 (60)	NS
BMI (kg/m^2)	27.13 ± 0.37	25.97 ± 0.67	24.22 ± 0.45	< 0.01
Hemoglobin (g/dL)	14.10 ± 0.16	14.51 ± 0.28	13.91 ± 0.11	NS
Platelet (× 10^3/mL)	245.5 ± 34.73	244.5 ± 13.8	260 ± 4.47	NS
WBC (k/UL)	6950 ± 319	7250 ± 330	7350 ± 146	NS
Neutrophil ratio	59.39% ± 1.20%	59.87% ± 1.20%	59% ± 1.1%	NS
Lymphocyte ratio	31.29% ± 1.09%	29.88% ± 1.10%	32% ± 0.78%	NS
RDW	14.28% ± 0.25%	13.37% ± 0.12%	12.96% ± 0.14%	< 0.01
MPV (fL)	8.63 ± 1.30	8.49 ± 1.06	8.20 ± 0.59	NS
MCV (fL)	86.34 ± 0.88	87.98 ± 1.37	85.5 ± 0.82	NS
ALT (IU/L)	54 ± 8.1	45 ± 3.97	32.5 ± 1.49	< 0.01
AST (IU/L)	40.05 ± 6.04	40.05 ± 3.01	24 ± 1.39	< 0.01
ALP (IU/L)	87.5 ± 9.09	81.5 ± 3.91	61 ± 1.87	< 0.01
GGT (IU/L)	61 ± 13.4	32 ± 3.4	27 ± 2.5	< 0.01
Albumin (g/dL)	4.36 ± 0.73	4.5 ± 0.42	4.9 ± 0.75	< 0.01
Creatinine	0.78 ± 0.033	0.79 ± 0.023	0.72 ± 0.014	NS
BUN (mg /dL)	12.25 ± 0.85	14.2 ± 0.67	13.46 ± 0.71	NS
Diabetes mellitus	19 (30.6)	9 (28.1)	0 (0)	NS
Hypertension	22 (35.4)	7 (21.8)	0 (0)	NS

Values are presented as *n* (%) frequencies, median ± SE for skewed distributed variables. BMI: Body-mass index; RDW: Red cell distribution width; MPV: Mean platelet volume; NS: Not significant; ALT: Alanine aminotransaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ glutamyl transferase; WBC: White blood cell; MCV: Mean corpuscle volume; BUN: Blood urine nitrogen.

healthy controls 18 (60%) were men ($P > 0.05$). The median RDW values were 14.28% ± 0.25%, 13.37% ± 0.12% and 12.96% ± 0.14%, respectively ($P < 0.01$). The clinical and laboratory data of patients with NASH, simple steatosis and healthy control groups are summarized in Table 1. The comparison of RDW values among NASH, simple steatosis and healthy control groups is also shown in Figure 2A.

Relationship between RDW and the presence of NASH and fibrosis

The ROC analysis suggested that a cutoff value of 13.56 has the highest sensitivity (61.3%) and specificity (72.6%) for detecting patients with NASH with an area under the curve (AUC) of 0.70 (95%CI: 0.600-0.805), ($P < 0.01$), as shown in Figure 3A.

According to the liver histopathological features, while the mild fibrosis group consisted of 40 patients, there were 22 patients in advanced fibrosis group. We compared RDW values between the mild and advanced fibrosis subgroups of NASH and found a statistically significant difference (13.95% ± 1.74% and 15.85% ± 1.89%, respectively, $P < 0.01$), as seen in Figure 2B. The ROC curve of RDW for the identification of advanced fibrosis in NASH was statistically significant (AUC = 0.78, 95%CI: 0.660 -0.903, $P < 0.01$), as shown in Figure 3B.

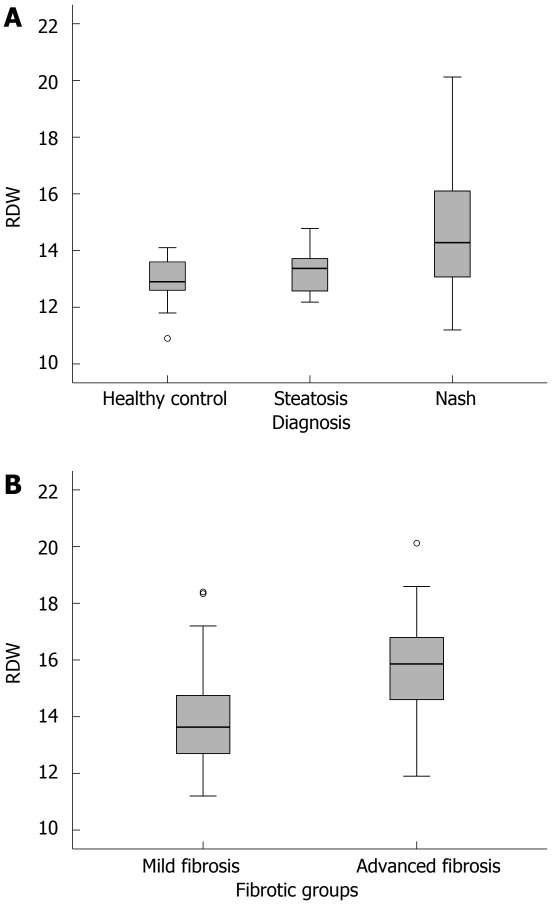

Figure 2 Red cell distribution width values of healthy controls, simple steatosis and non-alcoholic steatohepatitis groups (A), and fibrotic subgroups of non-alcoholic steatohepatitis (B). RDW: Red cell distribution width.

Correlation between RDW and clinical/histopathological features of patients with NASH

In Spearman correlation analysis, there were statistically significant correlations between RDW values and fibrotic scores, age and gender, as seen in Table 2. The correlation between fibrotic scores and RDW values reached statistically significance, such that Spearman's correlation coefficients were $r = 0.42$, and $P < 0.001$, as shown in Figure 4.

Univariate analysis for the presence of NASH indicated that age, gender, platelet counts, ALT, AST, ALP, GGT, albumin, BMI and RDW were statistically significant predictor factors of the presence of the NASH. The results of univariate analysis are shown in Table 3.

Those variables that were statistically significant in univariate logistic regression analyses were furthered subjected to multivariate logistic regression analysis, and RDW continued to be statistically significant and an independent predictor of fibrosis (OR = 1.75, 95%CI: 1.129-2.711, $P < 0.001$), as seen in Table 4.

DISCUSSION

In the present study, we concluded that patients with biopsy proven NASH have higher RDW values compared

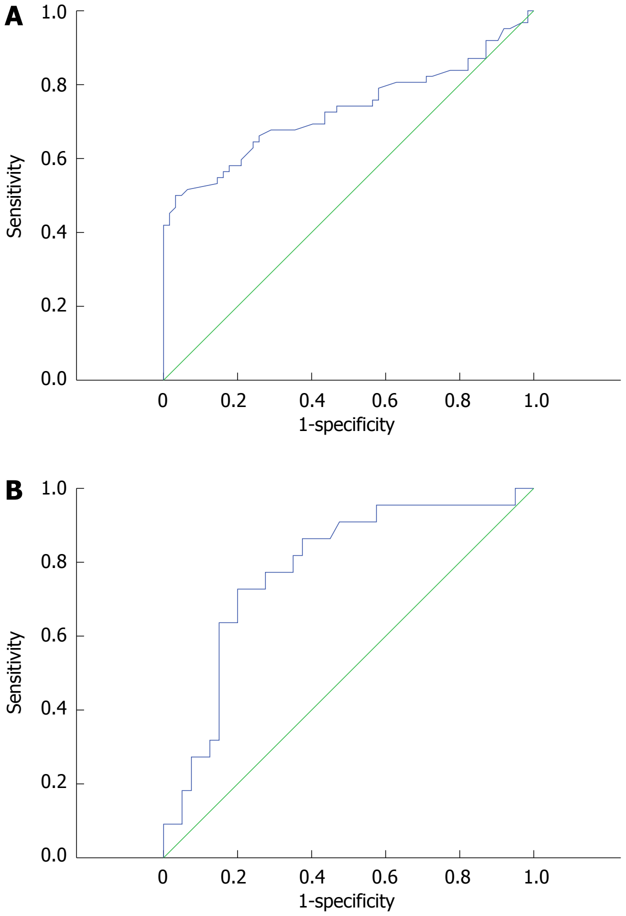

Figure 3 Receiver operating characteristics curve of red cell distribution width values for the identification of patients with non-alcoholic steatohepatitis (A), and the identification of fibrosis in non-alcoholic steatohepatitis (B).

Table 2 Red cell distribution width correlated with clinical characteristics and histological features of non-alcoholic steatohepatitis

Factor	*r*-value	*P*-value
Age (yr)	0.245	< 0.01
BMI (kg/m^2)	0.131	NS
Gender	-0.251	< 0.01
Fibrosis	0.579	< 0.01
Inflammation	0.207	NS
Steatosis	0.121	NS
Ballooning	0.175	NS
NASH	0.217	NS

BMI: Body mass index; RDW: Red cell distribution width; NASH: non-alcoholic steatohepatitis; NS: Not significant.

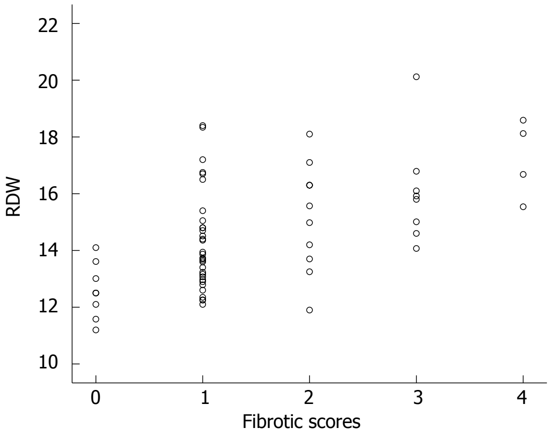

Figure 4 Correlation between red cell distribution width values and fibrotic scores of non-alcoholic steatohepatitis. RDW: Red cell distribution width. Score 0, *n* = 8; Score 1, *n* = 32; Score 2, *n* = 10; Score 3, *n* = 8; Score 4, *n* = 4.

with simple steatosis patients and healthy controls. The increase in RDW values correlated with disease progression because the patients with advanced fibrosis had higher RDW values than the mild fibrosis group. There was a positive correlation between RDW values and fibrotic scores. As a result of multivariate logistic regression analysis RDW was identified as an independent predictor of NASH and advanced liver fibrosis.

The search for a noninvasive diagnostic marker indicating the histological changes and fibrotic stages observed in NASH is very important. The absence of approved treatment methods for NASH means that care must be taken on the detection of advanced fibrosis. The risk stratification requires an adequate evaluation of fibrosis, which only currently possible by performing a liver biopsy. Biopsy is an invasive method with natural risks, costs, and is disturbing for the patients; therefore, considerable efforts have been made in the development of noninvasive alternatives of determining the degree of liver fibrosis.

Lou *et al*[21] found that RDW values were significantly increased in patients with hepatitis B and that high RDW values were associated with disease severity. Similarly Chen *et al*[22] concluded that RDW to platelet index is a routinely available and easily calculated index that could predict significant liver fibrosis caused by chronic hepatitis B with a high accuracy. They also estimated that this index may reduce the requirement of liver biopsy in patients infected with chronic hepatitis B. High RDW values are associated with poor outcomes in patients with cardiovascular diseases[23,24]. Although the main mechanism that causes elevation of RDW levels in these different conditions is unknown, it is speculated that inflammation may play an important role[25]. Some pathways may explain the increase in RDW values, such as impaired iron metabolism, suppressed erythropoietin gene expression, inhibition of proliferation of erythroid progenitor cells, downregulation of erythropoietin receptor expression and reduced erythrocyte circulatory half-life[26].

Alkhouri *et al*[27] found that the neutrophil to lymphocyte ratio was higher in patients with NASH and advanced fibrosis. They hypothesized that this ratio could be used as a novel noninvasive marker to predict advanced liver fibrosis caused by NASH. Tonelli *et al*[28] reported that higher RDW values might reflect an underlying chronic inflammation, which could result in an increased risk of cardiovascular disease.

These results suggest that inflammation may be a

Table 3 Univariate analyses for predicting liver fibrosis

	OR	95%CI	*P*-value
Age	1.05	1.004-1.109	< 0.05
Gender	1.46	0.516-4.171	NS
Platelet	0.98	0.988-0.996	< 0.01
ALT	1.08	0.976-1.004	NS
AST	0.98	0.963-1.007	NS
ALP	1.015	1.002-1.028	< 0.05
GGT	1.002	0.998-1.007	NS
RDW	1.73	1.242-2.409	< 0.01
Albumin	0.418	0.158-1.105	NS
BMI	1.012	0.847-1.209	NS

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ glutamyl aminotransferase; RDW: Red cell distribution width; BMI: Body mass index; NS: Not significant.

Table 4 Multivariate analyses for advanced liver fibrosis

	OR	95%CI	*P*-value
ALP	1.005	0.993-1.018	NS
RDW	1.75	1.129-2.711	< 0.05
Age	1.02	0.957-1.086	NS
Platelet	0.991	0.981-1.001	NS
Albumin	1.016	0.291-3.545	NS

ALP: Alkaline phosphatase; RDW: Red cell distribution width; NS: Not significant.

potential underlying biological mechanism for increased RDW values. Chronic inflammation may play a role in disease progression of NASH and fibrosis. Indeed, multiple inflammatory cytokines have been assessed in different studies as noninvasive markers for the presence of NASH and fibrosis[29]. Thus, increased RDW values may be considered as a chronic inflammatory process in the pathogenic basis of NASH. Moreover our findings revealed that RDW could discriminate fibrotic scores of NASH, making it a potentially useful marker for predicting the progression and fibrotic stages of NASH.

We hypothesized that the relationship between RDW and NASH may be a result of an effect of an inflammatory process that suppresses mature erythrocytes and secretes young erythrocytes into the circulation, leading to anisocytosis and high RDW values. To the best of our knowledge, this is the first study to evaluate an association between high RDW values and high fibrotic scores in a well-designed, specifically organized patient group of biopsy proven NASH.

There are some limitations to our study that: RDW was assessed on a single situation instead of multiple consecutive measurements, therefore we could not evaluate any biological variabilities and measurement errors that could be a result of this. Also erythropoietin, reticulocyte count, inflammatory markers such as interleukin (IL)-1, IL-6, tumor necrosis factor-α were not provided; they might provide some important information on the pathophysiology of underlying high RDW physiology. A lack of a marked fall in the platelet count of NASH patients may reflect the small sample size and small number of cirrhotic patients. This must be evaluated in large population based prospective studies.

In summary, our study showed that in patients with biopsy proven NASH, RDW is associated with histological severity and could be used to identify patients with advanced liver fibrosis. Unlike many other noninvasive markers of NAFLD, RDW is inexpensive, widely available and easily repeatable. Although the accuracy of RDW for detecting NASH and significant fibrosis is sufficient, RDW in combination with other markers may help to identify patients at increased risk of having advanced disease. Perhaps independently of anemia and other factors, RDW could be used to estimate the fibrotic process of different diseases. It may become one of the cornerstones of inflammation and fibrosis if confirmed in large population based studies in the future.

COMMENTS

Background

Non-alcoholic steatohepatitis (NASH) is one of the most common causes of chronic liver diseases. Liver biopsy is the only method for distinguishing NASH from other diagnoses and evaluating the damage and fibrosis of liver, but it may have procedure-related clinical complications.

Research frontiers

Therefore, there has been an attempt to find noninvasive methods for detecting the presence of NASH, liver fibrosis and avoiding procedure-related complications. In this study, the authors demonstrated that red cell distribution width (RDW) may indicate the presence of NASH and advanced liver fibrosis.

Innovations and breakthroughs

Recent reports have demonstrated that elevated RDW values were related to negative outcomes in cardiovascular and metabolic disorders, colon cancer and stroke independent of hemoglobin values. The association between RDW values and the severity of liver diseases has also been reported in two recent studies. This is the first study to demonstrate the importance of RDW in the diagnosis of NASH and predicting advanced liver fibrosis.

Applications

By understanding that high RDW values can be seen in the presence of NASH and advanced liver fibrosis, this study may represent a future strategy for non-invasive diagnostic method of patients with NASH.

Terminology

RDW is a widely available, inexpensive and easily repeatable parameter of variation of circulating red cells, reflecting the heterogeneity of red cell volume, a component of the complete blood count. Chronic inflammation plays a significant role in disease progression to NASH. Liver fibrosis is a result of chronic injury and can only be diagnosed invasively by liver biopsy.

Peer review

The results present a new laboratorial and non-invasive marker of NASH and fibrotic stage. The authors concluded that in patients with biopsy proven NASH, RDW is associated with histological severity and could be used to identify patients with advanced liver fibrosis disease. This result is interesting and RDW may be used as a non-invasive marker of indicating the presence of NASH and advanced liver fibrosis.

REFERENCES

1 **Brunt EM**. Nonalcoholic steatohepatitis: pathologic features and differential diagnosis. *Semin Diagn Pathol* 2005; **22**: 330-338 [PMID: 16939061]

2 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID:

15565570 DOI: 10.1002/hep.20466]
3 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-10 [PMID: 16540768]
4 **Lazo M**, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 2008; **28**: 339-350 [PMID: 18956290 DOI: 10.1055/s-0028-1091978]
5 **Zhou YJ**, Li YY, Nie YQ, Ma JX, Lu LG, Shi SL, Chen MH, Hu PJ. Prevalence of fatty liver disease and its risk factors in the population of South China. *World J Gastroenterol* 2007; **13**: 6419-6424 [PMID: 18081233]
6 **Zafrani ES**. Non-alcoholic fatty liver disease: an emerging pathological spectrum. *Virchows Arch* 2004; **444**: 3-12 [PMID: 14685853 DOI: 10.1007/s00428-003-0943-7]
7 **McCullough AJ**. Pathophysiology of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2006; **40** Suppl 1: S17-S29 [PMID: 16540762]
8 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]
9 **Day CP**. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 663-678 [PMID: 12406438]
10 **Feldstein AE**, Lopez R, Tamimi TA, Yerian L, Chung YM, Berk M, Zhang R, McIntyre TM, Hazen SL. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *J Lipid Res* 2010; **51**: 3046-3054 [PMID: 20631297 DOI: 10.1194/jlr.M007096]
11 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379 [PMID: 18510618 DOI: 10.1111/j.1572-0241.2007.01774.x]
12 **Felker GM**, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, Swedberg K, Wang D, Yusuf S, Michelson EL, Granger CB. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. *J Am Coll Cardiol* 2007; **50**: 40-47 [PMID: 17601544 DOI: 10.1016/j.jacc.2007.02.067]
13 **Ani C**, Ovbiagele B. Elevated red blood cell distribution width predicts mortality in persons with known stroke. *J Neurol Sci* 2009; **277**: 103-108 [PMID: 19028393 DOI: 10.1016/j.jns.2008.10.024]
14 **Spell DW**, Jones DV, Harper WF, David Bessman J. The value of a complete blood count in predicting cancer of the colon. *Cancer Detect Prev* 2004; **28**: 37-42 [PMID: 15041076 DOI: 10.1016/j.cdp.2003.10.002]
15 **Lee WS**, Kim TY. Relation between red blood cell distribution width and inflammatory biomarkers in rheumatoid arthritis. *Arch Pathol Lab Med* 2010; **134**: 505-506 [PMID: 20367302]
16 **Malandrino N**, Wu WC, Taveira TH, Whitlatch HB, Smith RJ. Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. *Diabetologia* 2012; **55**: 226-235 [PMID: 22002006 DOI: 10.1007/s00125-011-2331-1]
17 **Hu Z**, Sun Y, Wang Q, Han Z, Huang Y, Liu X, Ding C, Hu C, Qin Q, Deng A. Red blood cell distribution width is a potential prognostic index for liver disease. *Clin Chem Lab Med* 2013; **51**: 1403-1408 [PMID: 23314558 DOI: 10.1515/cclm-2012-0704]
18 **Milić S**, Mikolasević I, Radić M, Hauser G, Stimac D. Clinical utility of red cell distribution width in alcoholic and non-alcoholic liver cirrhosis. *Coll Antropol* 2011; **35** Suppl 2: 335-338 [PMID: 22220466]
19 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]
20 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]
21 **Lou Y**, Wang M, Mao W. Clinical usefulness of measuring red blood cell distribution width in patients with hepatitis B. *PLoS One* 2012; **7**: e37644 [PMID: 22649548 DOI: 10.1371/journal.pone.0037644]
22 **Chen B**, Ye B, Zhang J, Ying L, Chen Y. RDW to platelet ratio: a novel noninvasive index for predicting hepatic fibrosis and cirrhosis in chronic hepatitis B. *PLoS One* 2013; **8**: e68780 [PMID: 23874760 DOI: 10.1371/journal.pone.0068780]
23 **Hampole CV**, Mehrotra AK, Thenappan T, Gomberg-Maitland M, Shah SJ. Usefulness of red cell distribution width as a prognostic marker in pulmonary hypertension. *Am J Cardiol* 2009; **104**: 868-872 [PMID: 19733726 DOI: 10.1016/j.amjcard.2009.05.016]
24 **Cheng CK**, Chan J, Cembrowski GS, van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. *Lab Hematol* 2004; **10**: 42-53 [PMID: 15070217]
25 **Lippi G**, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* 2009; **133**: 628-632 [PMID: 19391664 DOI: 10.1043/1543-2165-133.4.628]
26 **Weiss G**, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; **352**: 1011-1023 [PMID: 15758012]
27 **Alkhouri N**, Morris-Stiff G, Campbell C, Lopez R, Tamimi TA, Yerian L, Zein NN, Feldstein AE. Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2012; **32**: 297-302 [PMID: 22097893]
28 **Tonelli M**, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M. Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. *Circulation* 2008; **117**: 163-168 [PMID: 18172029 DOI: 10.1161/CIRCULATIONAHA.107.727545]
29 **Wieckowska A**, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology* 2007; **46**: 582-589 [PMID: 17661414 DOI: 10.1002/hep.21768]

P- Reviewers: Castiella A, Ji G, Nakano S **S- Editor**: Zhai HH
L- Editor: Stewart GJ **E- Editor**: Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7419

World J Gastroenterol 2013 November 14; 19(42): 7419-7425
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Real-time virtual sonography visualization and its clinical application in biliopancreatic disease

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Received: June 13, 2013 Revised: August 14, 2013
Accepted: September 16, 2013
Published online: November 14, 2013

Abstract

AIM: To evaluate the usefulness of real-time virtual sonography (RVS) in biliary and pancreatic diseases.

METHODS: This study included 15 patients with biliary and pancreatic diseases. RVS can be used to observe an ultrasound image in real time by merging the ultrasound image with a multiplanar reconstruction computed tomography (CT) image, using pre-scanned CT volume data. The ultrasound used was EUB-8500 with a convex probe EUP-C514. The RVS images were evaluated based on 3 levels, namely, excellent, good and poor, by the displacement in position.

RESULTS: By combining the objectivity of CT with free scanning using RVS, it was possible to easily interpret the relationship between lesions and the surrounding organs as well as the position of vascular structures. The resulting evaluation levels of the RVS images were 12 excellent (pancreatic cancer, bile duct cancer, cholecystolithiasis and cholangiocellular carcinoma) and 3 good (pancreatic cancer and gallbladder cancer). Compared with conventional B-mode ultrasonography and CT, RVS images achieved a rate of 80% superior visualization and 20% better visualization.

CONCLUSION: RVS has potential usefulness in objective visualization and diagnosis in the field of biliary and pancreatic diseases.

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Key words: Biliary and pancreatic disease; Computed tomography-multiplanar reconstruction image; Navigation; Real-time ultrasound image; Real-time virtual sonography

Core tip: The real-time virtual sonography (RVS) system combined with ultrasonography (US) and computed tomography (CT) compensates for each of the deficiencies of US and CT. The visualization in biliary and pancreatic diseases using RVS added objectivity and detailed imaging for diagnosis lacking in conventional B-mode US and CT, and it becomes possible to make a more precise diagnosis. Moreover, it is possible to consider this system as providing images of detailed processes conveniently and in real time. RVS has potential usefulness in objective visualization and diagnosis in the field of biliary and pancreatic diseases.

Sofuni A, Itoi T, Itokawa F, Tsuchiya T, Kurihara T, Ishii K, Tsuji S, Ikeuchi N, Tanaka R, Umeda J, Tonozuka R, Honjo M, Mukai S, Moriyasu F. Real-time virtual sonography visualization and its clinical application in biliopancreatic disease. *World J Gastroenterol* 2013; 19(42): 7419-7425 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7419.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7419

INTRODUCTION

Recent advancements in technology have expanded the role of diagnosis by ultrasonography (US) in biliopancreatic diseases. Ultrasonography is useful because it is non-invasive and images can be obtained in real time. However, visualization is sometimes difficult in the presence of bone, gas and air, and thus has the problem of diminished objectivity. Real-time virtual sonography (RVS) (Hitachi-Aloka Medical Ltd., Tokyo, Japan) is a technological system that was developed in an attempt to resolve such problems.

This method merges ultrasound images with information from computed tomography (CT) images to produce the displayed image. In other words, the CT volume data is pre-stored into the device and the real-time ultrasound image is displayed simultaneously while the virtual view is reconstructed as a CT-multiplanar reconstruction (MPR) image from the stored volume data[1]. This is one method of compensating for the decreased objectivity in ultrasound diagnosis. This method is used during navigation in ultrasound-guided treatment and its usefulness has been reported in the field of liver disease[2-13]; however, no data has been presented in the field of biliopancreatic disease.

MATERIALS AND METHODS

Aim

The RVS system was applied on patients with biliopancreatic disease to investigate its usefulness and potential. The aim of the evaluation was to assess how the target lesion was correlated with the surrounding organs and blood vessels.

Materials

RVS was performed on 15 biliopancreatic patients at our department. Among the patients, 6 had pancreatic cancer, 1 had pancreatic endocrine tumor, 2 had gall bladder cancer, 4 had bile duct cancer, 1 had cholangiocellular carcinoma, and 1 had cholecystolithiasis.

Device

The RVS system (Figure 1) is made up of an ultrasound diagnostic device EUB-8500 (Hitachi-Aloka Medical Ltd., Tokyo, Japan), the EUP-C514 probe (Hitachi-Aloka Medical Ltd., Tokyo, Japan), which is a convex type, a personal computer (PC) and a magnetic position sensor unit to detect the position of the probe. Pre-scanned CT volume data is stored in a medium such as a compact disc read-only memory or PC using digital imaging and communication in medicine specifications *via* a local area network. The PC can display the images at a high speed of approximately 11 frames/s, with each frame being 256 × 256 pixels of virtual sonography. The PC is equipped with a magnetic position sensor unit to obtain information on the position of the probe. The magnetic position sensor unit consists of the main unit, a magnetism generator and a magnetic sensor. When the magnetism generator is placed close to the patient, the magnetic sensor attaches to the probe. On CT, by performing imaging of very thin slices (approximately 1 mm) by multidetector CT (MDCT) of at least 16 rows, the minimum volume data required to reconstruct MPR images can be collected. In our department, 32-row MDCT is used to collect volume data by 3-planar contrast imaging.

Obtaining information on probe position and its calibration

Information of the ultrasound probe position is obtained by the magnetic position sensor connected to the PC. The information obtained by this sensor is on its relative position to the magnetic sensor. Therefore, to prepare an MPR image that fits the ultrasound plane, it is necessary to decide on the pre-stored CT volume data and the position (reference point) of the ultrasound image. Ensiform cartilage that is palpable from the body surface is the ideal reference point. This is used to adjust the position (calibration). Apart from ensiform cartilage, calibration can also be performed using metal markers placed in the vasculature, kidneys, umbilical cord or body surface. In our department, calibration is mainly performed using ensiform cartilage. After the calibration, the ultrasound image obtained from the ultrasound scanning is displayed simultaneously. The virtual sonography-processing program in the PC detects the probe position and angle, reconstructs the corresponding planar image from the CT volume data, and displays it as an MPR image (Figure 2).

Definitions

The respective RVS images are evaluated based on 3 levels, namely, Excellent: hardy any displacement in position, Good: midway between Excellent and Poor, and Poor: major displacement in position. The RVS images were stored in video form and were evaluated by 3 gastroenterologists (Sofuni A, Itoi T, Itokawa F) with at least 8 years of experience in abdominal sonography evaluation.

RESULTS

The results were based on the decisions of the majority, and the result of an actual case is shown in Table 1. The resulting evaluation levels of the RVS images were 12 excellent (pancreatic cancer, bile duct cancer, cholecystolithiasis and cholangiocellular carcinoma) and 3 good (pancreatic cancer and gallbladder cancer). The evaluation of the RVS image in the patient with pancreatic endocrine tumor was excellent (Figure 3). The diagnosis by endoscopic ultrasound-guided fine needle aspiration was poorly differentiated neuroendocrine carcinoma. The relationship between the tumor and the celiac artery posed a problem in terms of choice of treatment. With the RVS image, the ultrasound showed no vascular irregularity, and the boundary between the tumor, celiac artery (CA) and splenic artery (SPA) remained intact, thus surgery was performed. Even when surgery was performed, there was

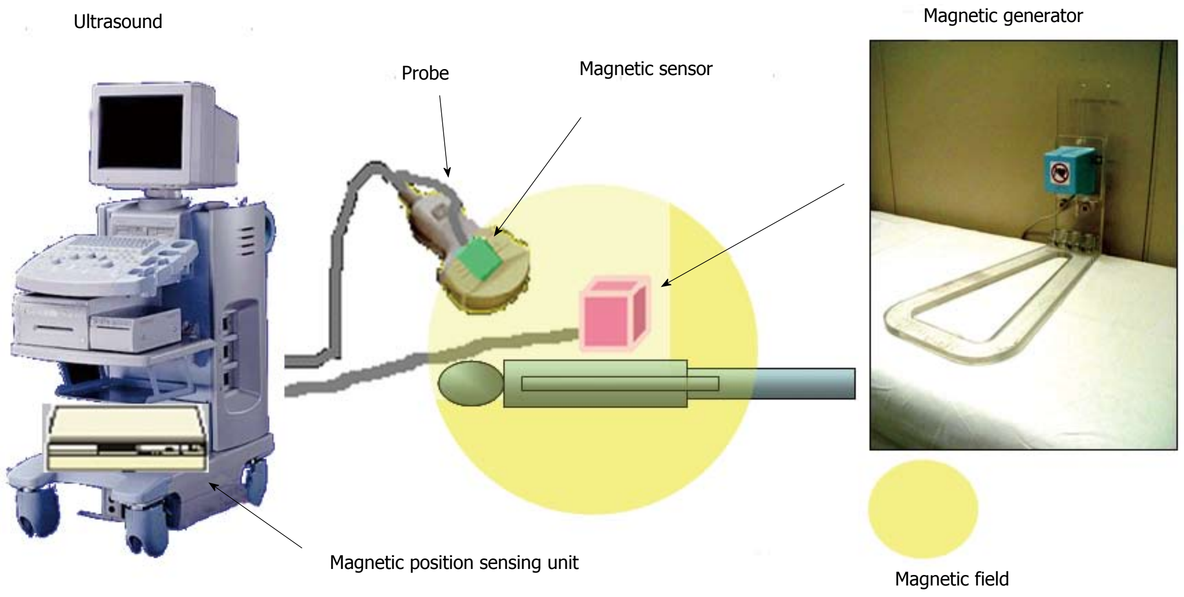


Figure 1 Real-time virtual sonography system. Pre-scanned computed tomography volume data are processed in the main body of the ultrasound machine.

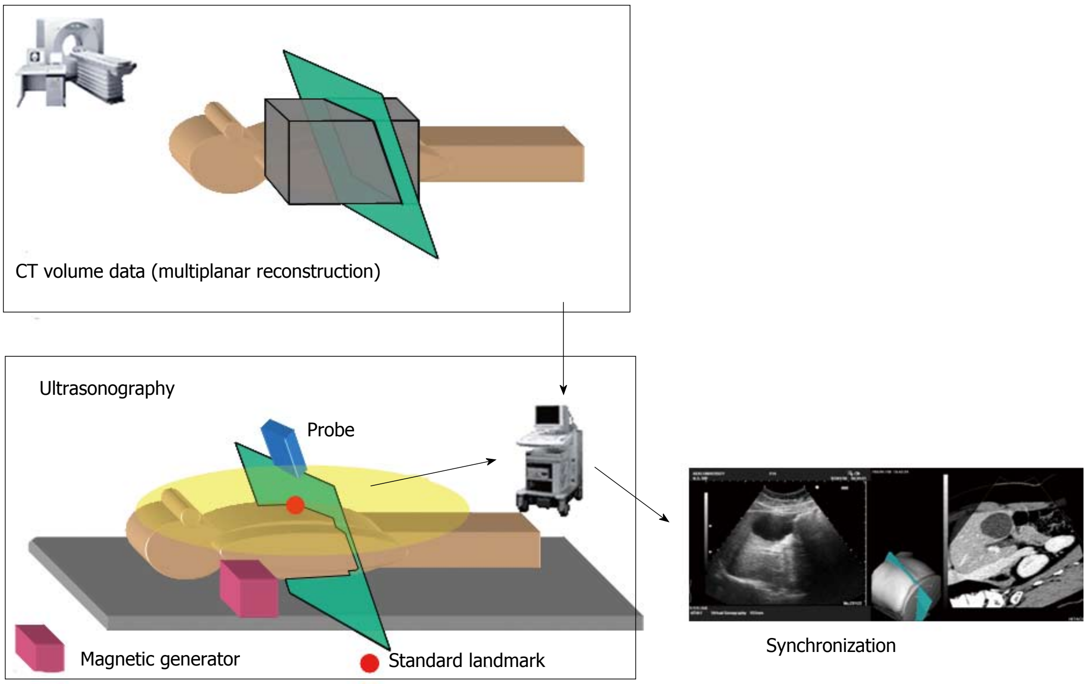


Figure 2 Calibration process. Transfer of the obtained computed tomography (CT) volume data; configuration of the settings for the standard landmark (ensiform cartilage/aorta/portal vein/other); and positional information of the probe is sensed. According to the probe sensor, the multiplanar reconstruction image is shown as an optimal angled plane.

no invasion into the arteries. With RVS, the positional relationship between the CA and the tumor could be evaluated objectively and was useful in judging whether or not surgery should be performed. Similarly, the positional relationship between the tumor mass and the vasculature could be assessed objectively, even in the cases where images of pancreatic cancer (Figure 4A, B) and bile duct cancer (Figure 4C-F) were judged as excellent. It was also useful in understanding the stage of progression and anatomical relationship. A good case (gall bladder cancer) is shown in Figure 4G.

DISCUSSION

US is a generally subjective examination and is considered to lack objectivity, particularly in the staging of malignant tumors. By combining the objectivity of CT with the obscureness in the free scanning of US, it was possible to easily interpret the relationship between the lesions and the surrounding organs, as well as the position of vascular structures. Based on the results, incorporation of the objectivity of CT into voluntary scanning with an ultrasound probe facilitated understanding of the positional

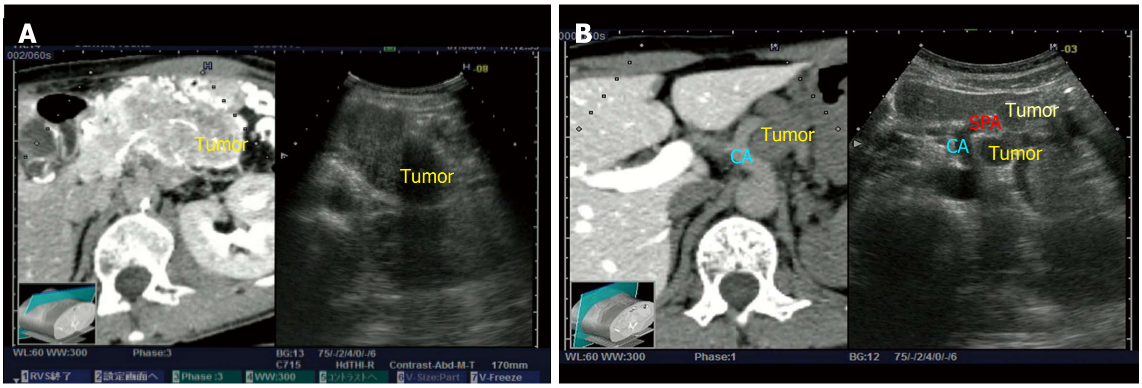


Figure 3 Pancreatic endocrine tumor (excellent case). A: Invasive pancreatic mass (60 mm) in body tail. B: Evaluation of the relationship between celiac artery (CA) and splenic artery (SPA): there was no vascular irregularity and the boundary between the tumor and CA/SPA remained intact.

Table 1 Characteristics of patients

No.	Case	Age (yr)	Sex	BMI (kg/m^2)	Breath-holding	Evaluation
1	Pancreatic cancer	76	M	18.1	Possible	Excellent
2	Pancreatic endocrine tumor	65	F	23.2	Possible	Excellent
3	Pancreatic cancer	73	F	21.3	Possible	Excellent
4	Pancreatic cancer	77	M	19.2	Possible	Excellent
5	Pancreatic cancer	65	M	22.2	Possible	Good
6	Pancreatic cancer	49	M	19.7	Possible	Excellent
7	Pancreatic cancer	58	F	21.4	Possible	Excellent
8	Bile duct cancer	81	F	12.7	Impossible	Excellent
9	Bile duct cancer	79	F	22.2	Possible	Excellent
10	Bile duct cancer	76	F	21.5	Possible	Excellent
11	Bile duct cancer	68	M	23.3	Possible	Excellent
12	Gallbladder cancer	80	M	25.1	Possible	Good
13	Gallbladder cancer	63	F	21.2	Possible	Good
14	Cholecystolithiasis	73	M	19.6	Possible	Excellent
15	Cholangiocellular carcinoma	68	F	21.7	Possible	Excellent

M: Male; F: Female; BMI: Body mass index.

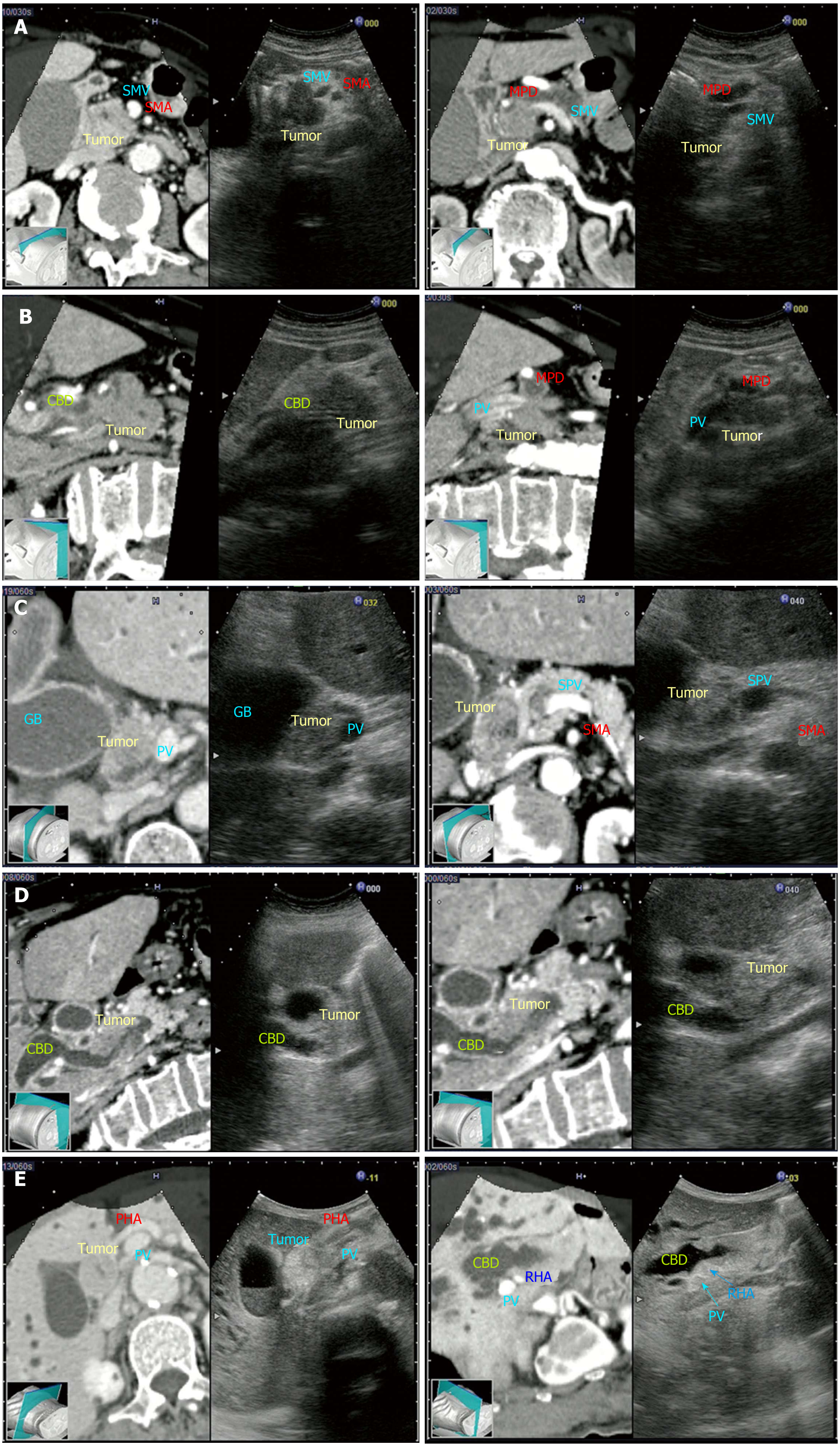
relationships among the lesion, the surrounding organs and vasculature, and the structure of the lesion itself. According to the study, this RVS system, combined with US and CT, also confirmed the comparatively precise diagnosis in US imaging. Moreover, this system compensates for each of the deficiencies of US and CT, and it becomes possible to provide more detailed information and make a more precise diagnosis.

US is usually more useful than CT in judging detailed imaging and the relationships with surrounding structures, and moreover, it is possible to consider the RVS system as providing images of detailed processes conveniently and in real time. For example, in Figure 3, it seems that the tumor invaded the blood vessel on CT imaging, but the RVS system showed that the tumor had not invaded the blood vessel. Therefore, RVS can aid in making a precise diagnosis when CT is unable to make judgments on the staging.

Compared with conventional B-mode US and CT, RVS images achieved a rate of 80% superior visualization and 20% better visualization, according to the statistics. The RVS visualization of lesions and the surrounding organs of all patients provided the objectivity and detailed imaging information for diagnosis lacking in conventional B-mode US and CT. It is also suggested to be useful when applied to making a diagnosis on the existence and stage of progression of malignant tumors, and the evaluation of infiltration into major blood vessels. Further studies in more patients are required to examine the applicability of this method to the diagnosis of malignant diseases and the extent of disease progression, including assessment of infiltration into major blood vessels. This needs to be investigated further in a larger number of patients.

Considering the investigations we have performed using RVS on patients in our department to date, the advantages and disadvantages of RVS are as outlined below. Five advantages are recognized at present: (1) CT objectivity in real-time sonographic images makes it easy to understand the structure and positional relationship between the lesion and the surrounding organs and vasculature; (2) it provides supportive education for surgeons and physicians that lack experience; (3) MPR images obtained by multi-slicing using the familiar operations of the ultrasound probe can be visualized instantly; (4) the display screen can be switched between the arterial phase, portal vein phase, and equilibrium phase (applies to the hemodynamics of the lesion and evaluation of the vasculature) by a simple operation; and (5) the common bile duct and pancreas are not affected by variations in breathing as much as the liver. There are 5 disadvantages: (1) the positions of the images during CT scanning and those of the US images are easily displaced due to breathing variations (liver > biliopancreatic area); (2) with only 1 reference point, synchronization of the position tends to be inadequate; (3) progression, infiltration and vascular evaluation are time-consuming and may put a strain on patients; (4) the focus is mainly on CT, thus US evaluation tends to be poor; and (5) the calibration is time-consuming. It is preferable to perform RVS after considering these advantages and disadvantages.

The main problem in the present RVS system is that when the position is displaced, calibration becomes time-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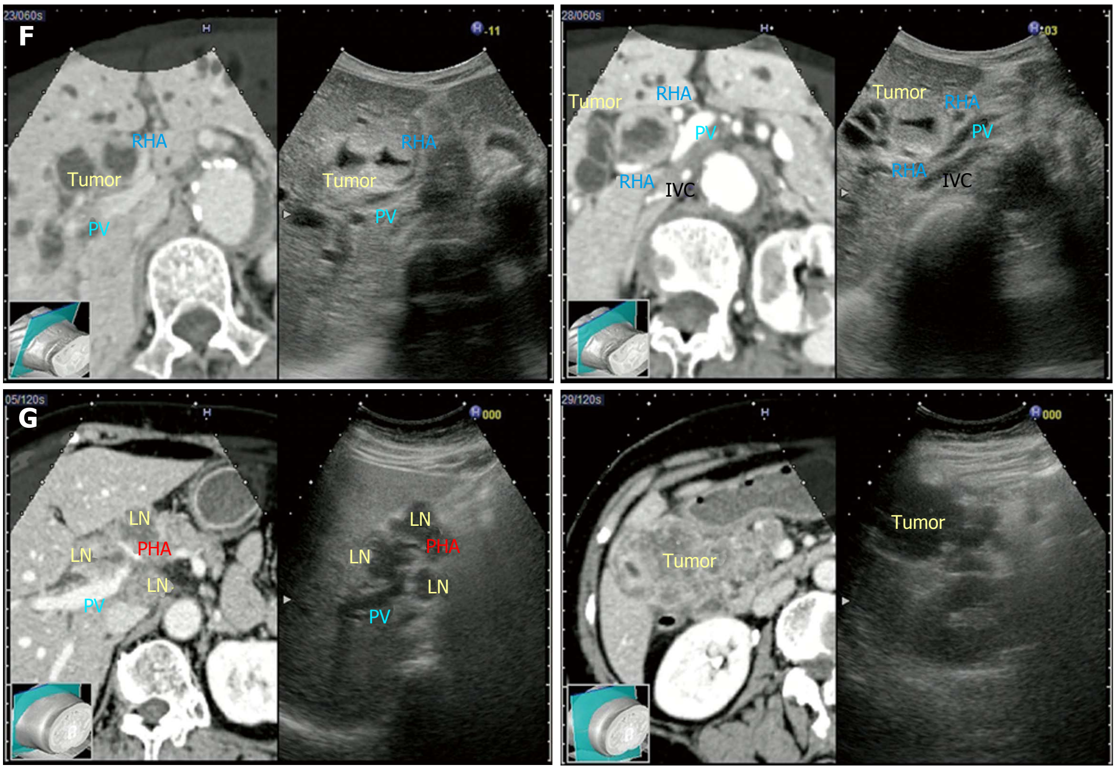

Figure 4 The resulting evaluation levels of the real-time virtual sonography images were 12 excellent (pancreatic cancer, bile duct cancer, cholecystolithiasis and cholangiocellular carcinoma) and 3 good (pancreatic cancer and gallbladder cancer). A: Pancreatic cancer (excellent case); the positional relationships among the tumor and vessels [superior mesenteric artery (SMA) and superior mesenteric vein, (SMV)] were well evaluated; B: Pancreatic cancer (excellent case); the positional relationships among the tumor, vessels [portal vein (PV)], common bile dilatation (CBD), and main pancreatic duct (MPD) were well evaluated; C: Bile duct cancer (excellent case); the positional relationships among the tumor and vessels (SMA, SMV and PV) were well evaluated; D: Bile duct cancer (excellent case); the positional relationship between the tumor and CBD was well evaluated; E: Bile duct cancer (excellent case); the positional relationships among the tumor and vessels [proper hepatic artery (PHA), right hepatic artery (RHA), PV] were well evaluated; F: Bile duct cancer (excellent case); the positional relationships among the tumor and vessels [RHA, PV and inferior vena cava (IVC)] were well evaluated; G: Gallbladder cancer (good case); there was a gap in the positional relationships among the tumor and vessels.

consuming. At Hitachi-Aloka Medical Ltd., an ultrasound phantom was prepared to examine the precision, and RVS was performed using simulated CT. As a result, displacement of the position of the slicing plane tended to depend on the distance between the magnetism generator and the magnetic sensor. However, the distance was reported to be approximately ± 5 mm within a range of 70 cm with the magnetism generator as the center. In addition, with only 1-point calibration, there is displacement of the position. Therefore, multiple-point calibration is important. Precision for calibration needs to be improved by taking these factors into consideration. Moreover, in actual clinical settings, the position of organs may also vary according to the posture and depth of respiration of the subject. Errors can also arise due to these factors; therefore, a corrective function for positional displacement as in the synchronization of breathing needs to be installed in the device. In addition, recent advances in computer and laser technologies have enabled the construction of MPR images of MDCT to be performed easily even with a notebook PC. Taking these points into consideration, one can say that the superiority of RVS is its potential in the areas of therapy and education.

Apart from diagnosis, RVS is now commonly used to assist treatment under ultrasound guidance in liver disease. Even in the biliopancreatic area, particularly with pancreatic cancer, focused ultrasound treatment and high intensity focused ultrasound (HIFU) treatment are now being performed[14]. In the current HIFU, treatment progresses while looking at the 2D ultrasound image. The application of this method allows easy understanding of the range of treatment, and safe treatment can be performed objectively.

This study suggests that RVS is useful when applied to making a diagnosis on the existence and stage of progression of malignant tumors, and making evaluations of infiltration into major blood vessels, in spite of some disadvantages. Further improvement and progress of RVS is expected in the field of therapy.

COMMENTS

Background

Ultrasonography (US) is non-invasive, and images can be obtained in real time. However, visualization is sometimes difficult with the presence of bone, gas and air, and thus has the problem of diminished objectivity. Real-time virtual sonography (RVS) is a technological system that was developed in an attempt to resolve such problems. This is one method of compensating for the decreased objectivity in ultrasound diagnosis. This method is used during navigation in ultrasound-guided treatment and its usefulness has been reported in the field of liver disease; however, no data has been presented in the field of biliopancreatic disease.

Research frontiers

The frontiers of research are in the fields of diagnosis and therapy for biliopancreatic disease.

Innovations and breakthroughs

The usefulness of the RVS system has been reported in the field of liver disease; however, no data has been presented in the field of biliopancreatic disease. RVS is useful when applied to making a precise diagnosis on the existence and stage of progression of malignant tumors, and making evaluations of infiltration into major blood vessels. Recently, therapies using ultrasound for biliopancreatic disease have been developed. Further improvement and progress of RVS is expected in the field of therapy.

Applications

The RVS system combined with US and computed tomography (CT) compensates for each of the deficiencies of US and CT, and it becomes possible to provide more detailed information and make a more precise diagnosis. Moreover, it is possible to consider the RVS system as providing images of detailed processes conveniently and in real time. Therefore, this system is useful in the case in which the detailed information and positional relationships among the lesions are needed for a precise diagnosis.

Terminology

RVS can be used to observe an ultrasound image in real time by merging the ultrasound image with a multiplanar reconstruction CT image, using prescanned CT volume data. The RVS system combined with US and CT compensates for each of the deficiencies of US and CT.

Peer review

This is a good study of the usefulness of RVS in biliary and pancreatic diseases. Twelve patients with biliary and pancreatic diseases in whom the combination of the objectivity of CT with free scanning using RVS was observed. This study showed that the visualization in biliary and pancreatic diseases using RVS had the objectivity and the detailed imaging for diagnosis lacking in conventional B-mode US and CT. This new technique has potential usefulness in visualizing and diagnosing biliary and pancreatic diseases in the future. Even though the patient numbers were small, the paper has the potential data to be used at the clinic for appropriate diagnosis.

REFERENCES

1 **Oshio K,** Shinmoto H. Simulation of US imaging by using a CT data set. *Radiology* 1996; **201** Suppl: 517. Available from: URL: http://serials.unibo.it/cgi-ser/start/en/spogli/df-s.tcl?prog_art=2201452&language=ENGLISH&view=articoli

2 **Minami Y**, Kudo M, Chung H, Inoue T, Takahashi S, Hatanaka K, Ueda T, Hagiwara H, Kitai S, Ueshima K, Fukunaga T, Shiozaki H. Percutaneous radiofrequency ablation of sonographically unidentifiable liver tumors. Feasibility and usefulness of a novel guiding technique with an integrated system of computed tomography and sonographic images. *Oncology* 2007; **72** Suppl 1: 111-116 [PMID: 18087191 DOI: 10.1159/000111716]

3 **Kawasoe H**, Eguchi Y, Mizuta T, Yasutake T, Ozaki I, Shimonishi T, Miyazaki K, Tamai T, Kato A, Kudo S, Fujimoto K. Radiofrequency ablation with the real-time virtual sonography system for treating hepatocellular carcinoma difficult to detect by ultrasonography. *J Clin Biochem Nutr* 2007; **40**: 66-72 [PMID: 18437215 DOI: 10.3164/jcbn.40.66]

4 **Kitada T**, Murakami T, Kuzushita N, Minamitani K, Nakajo K, Osuga K, Miyoshi E, Nakamura H, Kishino B, Tamura S, Hayashi N. Effectiveness of real-time virtual sonography-guided radiofrequency ablation treatment for patients with hepatocellular carcinomas. *Hepatol Res* 2008; **38**: 565-571 [PMID: 18218008 DOI: 10.1111/j.1872-034X.2007.00308.x]

5 **Crocetti L**, Lencioni R, Debeni S, See TC, Pina CD, Bartolozzi C. Targeting liver lesions for radiofrequency ablation: an experimental feasibility study using a CT-US fusion imaging system. *Invest Radiol* 2008; **43**: 33-39 [PMID: 18097275 DOI: 10.1097/RLI.0b013e31815597dc]

6 **Sandulescu L**, Saftoiu A, Dumitrescu D, Ciurea T. Real-time contrast-enhanced and real-time virtual sonography in the assessment of benign liver lesions. *J Gastrointestin Liver Dis* 2008; **17**: 475-478 [PMID: 19104714]

7 **Sandulescu L**, Saftoiu A, Dumitrescu D, Ciurea T. The role of real-time contrast-enhanced and real-time virtual sonography in the assessment of malignant liver lesions. *J Gastrointestin Liver Dis* 2009; **18**: 103-108 [PMID: 19337645]

8 **Nakai M**, Sato M, Sahara S, Takasaka I, Kawai N, Minamiguchi H, Tanihata H, Kimura M, Takeuchi N. Radiofrequency ablation assisted by real-time virtual sonography and CT for hepatocellular carcinoma undetectable by conventional sonography. *Cardiovasc Intervent Radiol* 2009; **32**: 62-69 [PMID: 18987911 DOI: 10.1007/s00270-008-9462-x]

9 **Okamoto E**, Sato S, Sanchez-Siles AA, Ishine J, Miyake T, Amano Y, Kinoshita Y. Evaluation of virtual CT sonography for enhanced detection of small hepatic nodules: a prospective pilot study. *AJR Am J Roentgenol* 2010; **194**: 1272-1278 [PMID: 20410414 DOI: 10.2214/AJR.08.2294]

10 **Kasuya K**, Sugimoto K, Kyo B, Nagakawa Y, Ikeda T, Mori Y, Wada T, Suzuki M, Nagai T, Itoi T, Shimazu M, Aoki T, Tsuchida A. Ultrasonography-guided hepatic tumor resection using a real-time virtual sonography with indocyanine green navigation (with videos). *J Hepatobiliary Pancreat Sci* 2011; **18**: 380-385 [PMID: 21127911]

11 **Liu FY**, Yu XL, Liang P, Cheng ZG, Han ZY, Dong BW, Zhang XH. Microwave ablation assisted by a real-time virtual navigation system for hepatocellular carcinoma undetectable by conventional ultrasonography. *Eur J Radiol* 2012; **81**: 1455-1459 [PMID: 21477961 DOI: 10.1016/j.ejrad.2011.03.057]

12 **Minami Y**, Kitai S, Kudo M. Treatment response assessment of radiofrequency ablation for hepatocellular carcinoma: usefulness of virtual CT sonography with magnetic navigation. *Eur J Radiol* 2012; **81**: e277-e280 [PMID: 21392916 DOI: 10.1016/j.ejrad.2011.02.026]

13 **Sandulescu DL**, Dumitrescu D, Rogoveanu I, Saftoiu A. Hybrid ultrasound imaging techniques (fusion imaging). *World J Gastroenterol* 2011; **17**: 49-52 [PMID: 21218083 DOI: 10.3748/wjg.v17.i1.49]

14 **Sofuni A**, Moriyasu F, Sano T, Yamada K, Itokawa F, Tsuchiya T, Tsuji S, Kurihara T, Ishii K, Itoi T. The current potential of high-intensity focused ultrasound for pancreatic carcinoma. *J Hepatobiliary Pancreat Sci* 2011; **18**: 295-303 [PMID: 21360084 DOI: 10.1007/s00534-010-0355-4]

P- Reviewers: Chowdhury P, Pezzilli R **S- Editor:** Gou SX
L- Editor: Cant MR **E- Editor:** Wu HL

WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7426

World J Gastroenterol 2013 November 14; 19(42): 7426-7432
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Hemorrhagic gastric and duodenal ulcers after the Great East Japan Earthquake Disaster

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Received: May 20, 2013 **Revised:** September 18, 2013
Accepted: September 29, 2013
Published online: November 14, 2013

Abstract

AIM: To elucidate the characteristics of hemorrhagic gastric/duodenal ulcers in a post-earthquake period within one medical district.

METHODS: Hemorrhagic gastric/duodenal ulcers in the Iwate Prefectural Kamaishi Hospital during the 6-mo period after the Great East Japan Earthquake Disaster were reviewed retrospectively. The subjects were 27 patients who visited our hospital with a chief complaint of hematemesis or hemorrhagic stool and were diagnosed as having hemorrhagic gastric/duodenal ulcers by upper gastrointestinal endoscopy during a 6-mo period starting on March 11, 2011. This period was divided into two phases: the acute stress phase, comprising the first month after the earthquake disaster, and the chronic stress phase, from the second through the sixth month. The following items were analyzed according to these phases: age, sex, sites and number of ulcers, peptic ulcer history, status of *Helicobacter pylori* (*H. pylori*) infection, intake of non-steroidal anti-inflammatory drugs, and degree of impact of the earthquake disaster.

RESULTS: In the acute stress phase from 10 d to 1 mo after the disaster, the number of patients increased rapidly, with a nearly equal male-to-female ratio, and the rate of multiple ulcers was significantly higher than in the previous year (88.9% *vs* 25%, $P < 0.005$). In the chronic stress phase starting 1 mo after the earthquake disaster, the number of patients decreased to a level similar to that of the previous year. There were more male patients during this period, and many patients tended to have a solitary ulcer. All patients with duodenal ulcers found in the acute stress phase were negative for serum *H. pylori* antibodies, and this was significantly different from the previous year's positive rate of 75% ($P < 0.05$).

CONCLUSION: Severe stress caused by an earthquake disaster may have affected the characteristics of hemorrhagic gastric/duodenal ulcers.

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Key words: Great East Japan Earthquake Disaster; Hemorrhagic gastric; Duodenal ulcer; *Helicobacter pylori* infection; Stress

Core tip: We determined the characteristics of hemorrhagic gastric/duodenal ulcers in the post-earthquake period within one medical district. We divided hemorrhagic gastric/duodenal ulcers into two groups, the acute stress phase group, consisting of the first month after the earthquake disaster, and the chronic stress phase group, from the second through the sixth month. We concluded that severe stress caused by this earthquake disaster exacerbated the characteristics of hemorrhagic gastric/duodenal ulcers.

Yamanaka K, Miyatani H, Yoshida Y, Asabe S, Yoshida T, Nakano M, Obara S, Endo H. Hemorrhagic gastric and duodenal ulcers after the Great East Japan Earthquake Disaster. *World J Gastroenterol* 2013; 19(42): 7426-7432 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7426.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7426

INTRODUCTION

Iwate Prefectural Kamaishi Hospital is located in the southern coastal area of Iwate Prefecture and is a regional core hospital with 15 departments and 272 beds in the Kamaishi medical district (Figure 1). This hospital is a core disaster hospital in the medical district, which has a population of approximately 55000. In the Great East Japan Earthquake Disaster that occurred on March 11, 2011, the Pacific coastal regions of East Japan suffered devastating damage from the earthquake itself and the consequent tsunami. Our hospital remained functional enough to accept all patients transported by ambulance from within the medical district, even after the disaster. The East Japan Earthquake Disaster significantly increased the occurrences of cardiovascular disease, respiratory disease, and cerebrovascular disease[1-9]. Since the number of patients visiting our hospital with hemorrhagic gastric/duodenal ulcers increased after this disaster, we compared those patients with the analogous patient population in 2010, the pre-earthquake period. This study aimed to elucidate the characteristics of ulcers in the post-earthquake period within our medical district.

MATERIALS AND METHODS

This study included 27 patients who visited our hospital with a chief complaint of hematemesis or hemorrhagic stool and were diagnosed as having hemorrhagic gastric/duodenal ulcers by upper gastrointestinal endoscopy during a 6-mo period starting on March 11, the day of the earthquake disaster, until September 10, 2011. This period was divided into 2 phases: the acute stress phase, comprising the first month after the earthquake disaster, and the chronic stress phase, from the second through the sixth month. The following items were analyzed according to these phases: age, sex, sites and number of ulcers, peptic ulcer history, status of *Helicobacter pylori* (*H. pylori*) infection, intake of non-steroidal anti-inflammatory drugs (NSAIDs), and degrees of impact of the earthquake disaster. Moreover, these data were compared with those obtained from 27 consecutive patients who visited our hospital with a chief complaint of hematemesis or hemorrhagic stool and were diagnosed as having hemorrhagic gastric/duodenal ulcers by upper gastrointestinal endoscopy during the same period of the previous year (March 11 to September 10, 2010). Rates of hemorrhagic gastric/duodenal ulcers reportedly increased after the earthquake disaster[10]. We performed endoscopy only on patients who presented with a chief complaint of hematemesis or hemorrhagic stool during the first few months after the earthquake disaster, so only patients with confirmed hemorrhagic gastric/duodenal ulcers were included in this study. The status of *H. pylori* infection was determined by serum antibody titer. If the antibody titer was more than 10 U/mL, the patient was considered *H. pylori* positive. The titers of two patients were not examined because they were immediately transferred to other hospitals.

RESULTS

Hemorrhagic gastric ulcer

No patients with hemorrhagic gastric ulcers visited our hospital during the first 10 d after the earthquake disaster (Table 1, Figure 2A).

Acute stress phase (the first month after the earthquake disaster): There were 9 patients with ulcers. Compared to the mean number of patients in the previous year (3.3 patients/mo in 2010), this number represented a marked increase of approximately 3-fold after the earthquake disaster, but the difference was not statistically significant (Table 2). The mean patient age was 68.3 years, similar to that of the previous year (69.2 years). The male-to-female ratio was 6:3. Although it was not significantly different from that of the previous year (15:5), the percentage of women was higher in the study period. Regarding ulcer morphology, multiple ulcers were observed in 8 patients (88.9%), and the incidence of this form was significantly higher than in the previous year (25%) ($P < 0.005$). The most common ulcer site was the gastric corpus, seen in 7 patients (77.8%), and the rate for this site was similar to that of the previous year (85%). Four patients had an ulcer history (44.4%), and the proportion of these patients was similar to that of the previous year (35%). Five patients were positive for serum *H. pylori* antibodies (55.6%), which was slightly lower than the previous year (64%) (not statistically significant).

Chronic stress phase (the second to the sixth month after the earthquake disaster): There were 11 patients with ulcers, with the mean number being 2.2 patients/mo, indicating a significant decrease compared to the number in the acute stress phase ($P < 0.05$). Moreover, the number in the chronic stress phase was smaller than that of the previous year (3.3 patients/mo) (not statistically significant). The mean patient age was 65.8 years, again similar to that of the previous year (69.2 years). The male-to-female ratio was 8:3, which was also similar to that of the previous year (15:5). Regarding ulcer morphology, multiple ulcers were observed in 5 patients (45.5%), which was a significantly lower rate than in the acute stress phase ($P < 0.05$). Moreover, the incidence

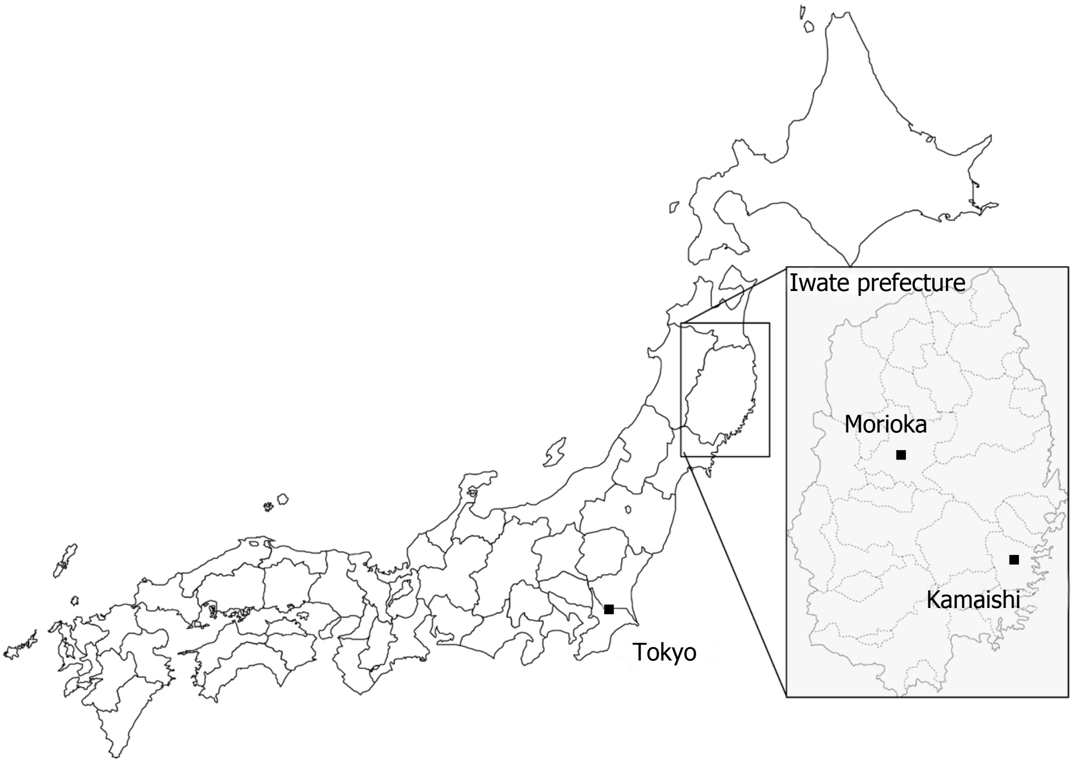

Figure 1 Location of Kamaishi city in Iwate prefecture in Japan.

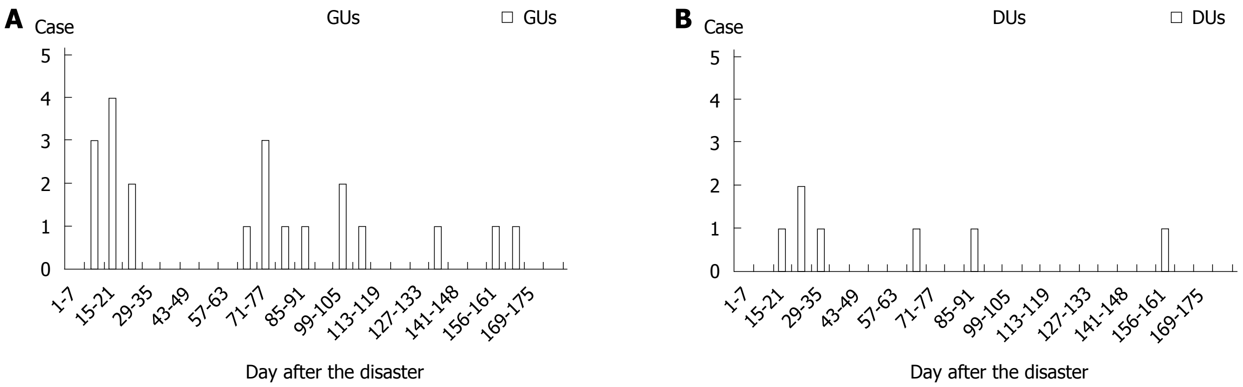

Figure 2 Incidence of hemorrhagic gastric (A) and duodenal (B) ulcers. GUs: Gastric ulcers; DUs: Duodenal ulcers.

in the chronic stress phase was nearly the same as that of the previous year (25%). Regarding ulcer site, ulcers were observed in the gastric corpus in 8 patients (72.7%), a rate that was similar to that of the previous year (85%). Three patients had an ulcer history (27.2%), and the proportion of these patients was nearly the same as that of the previous year (35%). Eight patients were positive for serum *H. pylori* antibodies (72.7%), and the proportion was similar to that of the previous year (64%).

Four of 6 patients who were negative for serum *H. pylori* antibodies used neither oral NSAIDs nor steroids at any time during the study period.

Hemorrhagic duodenal ulcer

No patients with hemorrhagic duodenal ulcers visited our hospital during the first 19 d after the earthquake disaster (Table 3, Figure 2B).

Acute stress phase (the first month after the earthquake disaster): Three patients had ulcers. Compared to the mean number of patients in the previous year (1.2 patients/mo), this number was higher after the earthquake disaster, though it was not statistically significant (Table 2). The mean patient age was 68.6 years, similar to that of the previous year (57.3 years). The male-to-female ratio was 2:1, which was almost the same as that of the previous year (5:2). All patients showed multiple ulcers on the posterior wall. None of the patients had an ulcer history, which was lower than the previous year (75%), though not significantly. All patients were negative for serum *H. pylori* antibodies, and this was significantly different from the previous year (positive rate: 75%) ($P < 0.05$).

Table 1 Details of patients with gastric ulcers in 2011

Day	Age (yr)	Sex	Location	Number	p.h	*Helicobacter pylori* antibody titer	Property damage	Dead of family
Acute stage								
11	52	M	U	Multiple	-	+ 17.4	Complete	-
13	67	M	M	Multiple	+	- 6.6	Complete	-
13	64	F	L	Multiple	-	- < 3.0	-	Grandchild
17	80	F	M	Multiple	-	- < 3.0	-	Daughter
17	80	F	M	Multiple	+	+ unknown	Partial	-
17	61	F	M	Multiple	+	+ 31.0	-	-
21	67	M	M	Multiple	+	+ 18.6	-	-
25	62	M	M	Single	-	+ 16.3	Complete	-
27	82	M	M	Multiple	-	- 6.5	Partial	-
Chronic stage								
66	76	F	M	Multiple	+	- < 3.0	Complete	-
72	73	M	U	Multiple	-	+ 22.7	Complete	-
76	59	M	U	Single	-	+ 15.5	Complete	-
77	54	M	M	Single	-	+ 22.1	-	-
83	75	F	M	Single	-	+ 33.3	Complete	-
99	49	M	M	Single	-	- 5.2	-	-
101	67	M	M	Multiple	-	- 8.9	-	-
110	57	M	M	Multiple	+	+ 12.1	Complete	-
136	75	M	M	Single	-	+ unknown	-	-
158	82	F	U	Multiple	+	+ 27.4	-	-
164	54	M	M	Single	-	+ 25.3	Complete	Daughter

p.h.: Past history of peptic ulcer; U: Upper lesion; M: Middle lesion; L: Lower lesion.

Table 2 Characteristics of patients with gastric ulcers and duodenal ulcers

	2011 acute stage	2011 chronic stage	2010	*P* value
GUs				
Number of patients (*n*)	9	11	20	NS
Average number of patient per month	9	2.2	3.3	$< 0.05^{a}$
Age (yr)	68.3	65.5	69.2	NS
Male/female ratio	1.3 (5/4)	2.7 (8/3)	3 (15/5)	NS
Simple/multiple ratio	0.13 (1/8)	1.2 (6/5)	3 (15/5)	$< 0.05^{a} < 0.005^{d}$
Helicobacter pylori (+)/(-)	6/3	8/3	7/4 (unknown 9)	NS
DUs				
Number of patients (*n*)	3	4	7	NS
Average number of patients per month	3	0.8	1.2	NS
Age (yr)	68.6	56.3	57.3	NS
Male/female ratio	2 (2/1)	3 (3/1)	2.5 (5/2)	NS
Simple/multiple ratio	0.5 (1/2)	3 (3/1)	1.3 (4/3)	NS
Helicobacter pylori (+)/(-)	0/3	1/3	4/2 (unknown 1)	$< 0.05^{a}$

GUs: Gastric ulcers; DUs: Duodenal ulcers. $^{a}P < 0.05$ between 2011 acute stage *vs* 2011 chronic stage; $^{c}P < 0.05$, $^{d}P < 0.01$ between 2011 acute stage *vs* 2010.

Chronic stress phase (the second to the sixth month after the earthquake disaster): Four patients had ulcers, and the mean number of 0.8 patient/mo was similar to that of the previous year (1.2 patients/mo). The mean patient age was 56.3 years, again similar to that of the previous year (57.3 years). The male-to-female ratio was 3:1, which was also quite similar to that of the previous year (5:2). Although 4 patients (100%) had an ulcer history, there was no significant difference from the previous year (43%). Three patients were positive for serum *H. pylori* antibodies (75%), and this proportion was similar to that of the previous year (67%).

DISCUSSION

Both *H. pylori* infection and NSAIDs have major roles in the pathogenesis of peptic ulcers[11-16]. Stress is also considered an important causal factor in peptic ulcer disease[17], and there are studies on the involvement of *H. pylori* infection and stress in the incidence of peptic ulcers after the Great Hanshin-Awaji Earthquake Disaster in 1995[18-20].

In the Great East Japan Earthquake Disaster that occurred on March 11, 2011, the Kamaishi medical district (population of approximately 55000), where our hospital is located, also suffered serious damage due to the tsunami, and the combined number of dead and missing persons was approximately 2300. Many disaster victims also lost their homes. They were forced to stay at shelters or to live without adequate infrastructure, such as gas, water, and electricity supplies. These disaster victims experienced severe and unprecedented stress,

Table 3 Details of patients with duodenal ulcers in 2011

Day	Age (yr)	Sex	Location	Number	p.h.	*Helicobacter pylori* antibody titer	Property damage	Dead family
Acute stage								
19	85	M	AW/PW	Multiple	-	- < 3.0	Complete	Wife
23	51	M	PW	Single	-	- < 3.0	Complete	-
25	70	F	PW	Multiple	-	- < 3.0	Complete	Son
Chronic stage								
35	59	M	PW	Single	+	-3.4	Complete	-
65	72	F	AW/PW	Multiple	+	10.3	-	-
87	59	M	PW	Single	+	15.5	-	-
159	35	M	AW	Single	+	34.3	-	-

p.h.: Past history of peptic ulcer; AW: Anterior wall; PW: Posterior wall.

which persisted for a considerable period of time. In this study, the acute stress phase was defined as the first month, when numerous aftershocks occurred and when the disaster victims lived under extremely severe stress due to poor living conditions. The chronic stress phase was defined as the period from the second through sixth month after the earthquake disaster. We analyzed the clinical and demographic data of ulcer patients for each period.

Because the emergency transport routes were restored the day after the earthquake, many patients were transported to our hospital immediately after this disaster. Nevertheless, the day when the first patient visited our hospital with a chief complaint of hematemesis or hemorrhagic stool after the earthquake was March 22, 11 d after the disaster. Subsequently, the number of patients visiting our hospital with one of these chief complaints gradually increased, and 12 patients (mean: 3 patients/wk) with hemorrhagic gastric/duodenal ulcer visited in the first month. Because the mean number of patients with hemorrhagic gastric/duodenal ulcers for a period of 6 mo starting March 11, 2010, had been 1.13 patients/wk, there was an apparent increase after the March 11, 2011 earthquake disaster. However, the number rapidly decreased after the first month, and the subsequent mean number was 0.75 patient/wk, which was slightly lower than that of the previous year.

Matsuura *et al*[21] studied upper gastrointestinal bleeding after the Great East Japan Earthquake Disaster and found that there were few patients immediately after the earthquake disaster but that the number increased from a few days to 1 mo after the disaster. According to a study conducted in 1998 on peptic ulcers occurring after the Great Hanshin-Awaji Earthquake Disaster[18], Aoyama *et al*[18] also reported that the number of patients decreased during the first 2 wk after the earthquake disaster as compared to the previous year, then increased during the third through fourth weeks, and returned to a similar level to that of the previous year from the fifth through sixth weeks. Takakura *et al*[22] also reported that the number of patients with gastric ulcers increased by 4-fold 2 wk after the Great Hanshin-Awaji Earthquake Disaster, gradually decreased thereafter, and it had returned to near the level of the previous year 2.5 mo later. Our data show a similar trend to these reports. The increase in the number of patients during the acute stress phase strongly suggests that severe stress due to the violent earthquake and consequent tsunami contributed to the ulcer incidence. We have no clear explanation why the incidence of hemorrhagic gastric/duodenal ulcers was low during the first 10 d after the earthquake disaster, a time when the disaster victims were under extremely severe stresses concerning matters of life and death. It was assumed that stressful conditions might need to persist for a certain period of time before the breakdown of biological defense mechanisms.

The male-to-female ratio of gastric ulcer patients was 6:3. However, the ratio in the chronic stress phase was 8:3, a higher percentage of men. The examination of the data obtained during the same period in 2010 at our hospital also shows that the male-to-female ratio was 15:5, indicating a trend for a much higher percentage of men. Moreover, a study conducted in Japan found that the male-to-female ratio among peptic ulcer patients was 71:29, indicating that this disease occurs approximately 2.4 times more frequently in men than in women[23]. Based on a comparison of these data, an approximately equal incidence between men and women might be one of the characteristics of gastric ulcer presumably caused by acute stress after an earthquake disaster. Meanwhile, the male-to-female ratios for duodenal ulcer were 2:1 in the acute stress phase and 3:1 even in the chronic stress phase. These ratios were essentially consistent with the trends observed in most studies, and we assume that there are gender differences in the incidence of gastric and duodenal ulcers caused by acute stress.

The mean age of patients with gastric ulcers was 68.3 or 65.5 years old in the acute stress phase or chronic stress phase, respectively, which were not significantly different. The mean ages of those with duodenal ulcers were 68.6 and 56.3 years, respectively, indicating that the patients tended to be older in the acute stress phase.

Regarding the number of gastric ulcer lesions, multiple ulcers were observed in 8 (88.9%) of 9 patients in the acute stress phase. On the other hand, multiple ulcers were observed in 5 (45.5%) of 11 patients in the chronic stress phase, and an increase in the number of patients with a solitary ulcer was documented. Regarding the number of duodenal ulcer lesions, multiple ulcers were observed in 2 (66.7%) of 3 patients in the acute stress phase. In the chronic stress phase, only 1 (25%) of 4 patients had multiple ulcers. According to the data on patients at our hospital during the same period in 2010, 5 (25%) of 20 patients had multiple gastric ulcers, and 3 (42.9%) of 7 patients had multiple duodenal ulcers. Thus, both hemorrhagic gastric and duodenal ulcers in the acute stress phase were often multiple. Kanno *et al*[24] reported that post-disaster hemorrhagic ulcers were frequently observed in the stomach as multiple ulcers at the same time.

Five (55.6%) of 9 patients in the acute stress phase and 8 (72.7%) of 11 patients in the chronic stress phase were positive for *H. pylori* antibodies. All patients of hemorrhagic gastric/duodenal ulcers had no history of eradication therapy of *H. pylori*. During the same period in 2010 (excluding unknown cases), 7 (63.6%) of 11 patients were *H. pylori* positive, a number similar to that after the earthquake disaster. In contrast, none of the 3 patients with duodenal ulcers was positive for *H. pylori* antibodies in the acute stress phase, and this incidence was significantly lower than that of the chronic stress phase, in which 4 (100%) of 4 patients were positive. During the same period in 2010, 4 (66.7%) of 6 patients with duodenal ulcers were positive for *H. pylori* antibodies. The rate of peptic ulcer not associated with the use of drugs, such as NSAIDs, in Japanese patients negative for *H. pylori* infection was reported by Aoyama *et al*[25] to be 1.9% to 5.1% and by Nishikawa *et al*[26] to be 1.3%. However, Kanno *et al*[10] reported that patients with non-*H. pylori* and non-NSAID gastroduodenal ulcers accounted for as many as 24% of cases after the Great East Japan Earthquake Disaster, and their analysis showed that there were many cases with ulceration attributable to stress. In this study, 8 (29.6%) of 27 patients, including those with gastric/duodenal ulcers, were negative for *H. pylori* antibodies and had not taken oral NSAIDs. The incidence of such cases was extremely high. All 8 patients were experiencing overwhelming stress. For example, they had dead or missing family members or were forced to stay at shelters due to the complete destruction of their homes. This raises the possibility that hemorrhagic gastric/duodenal ulcers can be caused by stress alone.

We did not encounter patients with hemorrhagic acute gastric mucosal lesion that might have been induced by primary infection by *H. pylori*. There is a minor possibility that those patients got acutely infected by *H. pylori* and were not yet serologically positive. However, no incident of waterborne infection was reported in this area during the period, and the quality of most sources of drinking water was maintained in a hygienic state. We do not think that a majority of them got infected by *H. pylori* during the study period.

As to the location of hemorrhagic duodenal ulcers, all patients had ulcers in the posterior wall of the bulbus in the acute stress phase. The proportion of these patients was higher than in the chronic stress phase and the previous year. Although the difference between these proportions was not statistically significant, stress-induced duodenal ulcers without *H. pylori* infection may tend to occur in the posterior wall of the bulbus.

Our study has some limitations. This was an observational analysis that was performed on a limited closed lesion with a small sample size. Under the confused and extraordinary circumstances in the aftermath of the great disaster, detailed examinations were difficult to perform. Endoscopic examinations were mainly performed for hemorrhagic gastric/duodenal ulcers. Therefore, the pathophysiology and incidence of non-hemorrhagic and asymptomatic peptic ulcers are unknown. However, we expect that this study has some useful information for the care of hemorrhagic gastro/duodenal injury at times of great disasters.

In conclusion, severe stress caused by an earthquake disaster may have affected the characteristics of hemorrhagic gastric/duodenal ulcers.

COMMENTS

Background

Under severe stress, the incidence of hemorrhagic gastric/duodenal ulcers increases. However, there are few reports on the incidence and characteristics of hemorrhagic gastric/duodenal ulcers after a great earthquake disaster. Since the number of patients visiting our hospital with hemorrhagic gastric/duodenal ulcers increased after the Great East Japan Earthquake Disaster, the authors compared those patients with hemorrhagic gastric/duodenal ulcer patients in 2010, the pre-earthquake period. This study aimed to elucidate the characteristics of ulcers in the post-earthquake period within one medical district.

Research frontiers

It is not well known whether severe stress independently causes hemorrhagic gastric/duodenal ulcers without *Helicobacter pylori* (*H. pylori*) infection and non-steroidal anti-inflammatory drug (NSAID) use.

Innovations and breakthrough

In this medical district, all patients who developed gastric/duodenal ulcers were admitted to one hospital. Therefore, interesting differences in the characteristics of hemorrhagic ulcers before and after the earthquake disaster were observed. Moreover, it is presumed that severe stress caused by this earthquake disaster may have affected the characteristics of hemorrhagic gastric/duodenal ulcers without *H. pylori* infection and NSAIDs.

Applications

This study will provide some useful information for the care of hemorrhagic gastro/duodenal injuries at the time of a great disaster.

Terminology

The Great East Japan Earthquake Disaster occurred on March 11, 2011. The Pacific coastal regions of East Japan suffered devastating damage by the earthquake itself and the consequent tsunami.

Peer review

This is an important study in which dynamic changes in the characteristics of complicated peptic ulcer patients is depicted. Taking care of patients and performing such an analysis in a devastating circumstance is encouraging to all.

REFERENCES

1 **Nakamura A**, Satake H, Abe A, Kagaya Y, Kohzu K, Sato K, Nakajima S, Fukui S, Endo H, Takahashi T, Nozaki E, Tamaki K. Characteristics of heart failure associated with the Great East Japan Earthquake. *J Cardiol* 2013; **62**: 25-30 [PMID: 23623140 DOI: 10.1016/j.jjcc.2013.02.015]

2 **Yamauchi H**, Yoshihisa A, Iwaya S, Owada T, Sato T, Suzuki S, Yamaki T, Sugimoto K, Kunii H, Nakazato K, Suzuki H, Saitoh S, Takeishi Y. Clinical features of patients with de-

compensated heart failure after the Great East Japan Earthquake. *Am J Cardiol* 2013; **112**: 94-99 [PMID: 23561586 DOI: 10.1016/j.amjcard.2013.02.057]

3 **Aoki T**, Takahashi J, Fukumoto Y, Yasuda S, Ito K, Miyata S, Shinozaki T, Inoue K, Yagi T, Komaru T, Katahira Y, Obata A, Hiramoto T, Sukegawa H, Ogata K, Shimokawa H. Effect of the Great East Japan Earthquake on cardiovascular diseases--report from the 10 hospitals in the disaster area. *Circ J* 2013; **77**: 490-493 [PMID: 23328448 DOI: 10.1253/circj.CJ-12-1594]

4 **Aoki T**, Fukumoto Y, Yasuda S, Sakata Y, Ito K, Takahashi J, Miyata S, Tsuji I, Shimokawa H. The Great East Japan Earthquake Disaster and cardiovascular diseases. *Eur Heart J* 2012; **33**: 2796-2803 [PMID: 22930461 DOI: 10.1093/eurheartj/ehs288]

5 **Yamanda S**, Hanagama M, Kobayashi S, Satou H, Tokuda S, Niu K, Yanai M. The impact of the 2011 Great East Japan Earthquake on hospitalisation for respiratory disease in a rapidly aging society: a retrospective descriptive and cross-sectional study at the disaster base hospital in Ishinomaki. *BMJ Open* 2013; **3**: pii: e000865 [PMID: 23293238]

6 **Aoyagi T**, Yamada M, Kunishima H, Tokuda K, Yano H, Ishibashi N, Hatta M, Endo S, Arai K, Inomata S, Gu Y, Kanamori H, Kitagawa M, Hirakata Y, Kaku M. Characteristics of infectious diseases in hospitalized patients during the early phase after the 2011 great East Japan earthquake: pneumonia as a significant reason for hospital care. *Chest* 2013; **143**: 349-356 [PMID: 22911275 DOI: 10.1378/chest.11-3298]

7 **Nakadate T**, Nakamura Y, Yamauchi K, Endo S. Two cases of severe pneumonia after the 2011 Great East Japan Earthquake. *Western Pac Surveill Response J* 2012; **3**: 67-70 [PMID: 23908944]

8 **Kobayashi S**, Hanagama M, Yamanda S, Satoh H, Tokuda S, Kobayashi M, Ueda S, Suzuki S, Yanai M. The impact of a large-scale natural disaster on patients with chronic obstructive pulmonary disease: the aftermath of the 2011 Great East Japan Earthquake. *Respir Investig* 2013; **51**: 17-23 [PMID: 23561254 DOI: 10.1016/j.resinv.2012.10.004]

9 **Omama S**, Yoshida Y, Ogasawara K, Ogawa A, Ishibashi Y, Nakamura M, Tanno K, Ohsawa M, Onoda T, Itai K, Sakata K. Influence of the great East Japan earthquake and tsunami 2011 on occurrence of cerebrovascular diseases in Iwate, Japan. *Stroke* 2013; **44**: 1518-1524 [PMID: 23640824 DOI: 10.1161/STROKEAHA.111.000442]

10 **Kanno T**, Iijima K, Abe Y, Koike T, Shimada N, Hoshi T, Sano N, Ohyauchi M, Ito H, Atsumi T, Konishi H, Asonuma S, Shimosegawa T. Peptic ulcers after the Great East Japan earthquake and tsunami: possible existence of psychosocial stress ulcers in humans. *J Gastroenterol* 2013; **48**: 483-490 [PMID: 23053423 DOI: 10.1007/s00535-012-0681-1]

11 **Asaka M**, Kato M, Kudo M, Meguro T, Kimura T, Miyazaki T, Inoue K. The role of Helicobacter pylori in peptic ulcer disease. *Gastroenterol Jpn* 1993; **28** Suppl 5: 163-167 [PMID: 8359625 DOI: 10.1007/BF02989228]

12 **Huang JQ**, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002; **359**: 14-22 [PMID: 11809181]

13 **Shiotani A**, Sakakibara T, Yamanaka Y, Imamura H, Tarumi K, Manabe N, Kamada T, Kusunoki H, Hata J, Haruma K. Upper gastrointestinal ulcer in Japanese patients taking low-dose aspirin. *J Gastroenterol* 2009; **44**: 126-131 [PMID: 19214674 DOI: 10.1007/s00535-008-2290-6]

14 **Okada K**, Inamori M, Imajo K, Chiba H, Nonaka T, Shiba T, Sakaguchi T, Atsukawa K, Takahashi H, Hoshino E, Nakajima A. Clinical study of upper gastrointestinal bleeding associated with low-dose aspirin in Japanese patients. *Hepatogastroenterology* 2009; **56**: 1665-1669 [PMID: 20214214]

15 **Iijima K**, Ara N, Abe Y, Koike T, Iwai W, Uno K, Asano N, Imatani A, Ohara S, Shimosegawa T. Gastric acid secretion level modulates the association between Helicobacter pylori infection and low-dose aspirin-induced gastropathy. *J Gastroenterol* 2011; **46**: 612-619 [PMID: 21359520 DOI: 10.1007/s00535-011-0385-y]

16 **Nadatani Y**, Watanabe T, Tanigawa T, Sogawa M, Yamagami H, Shiba M, Watanabe K, Tominaga K, Fujiwara Y, Yoshiyama M, Arakawa T. Incidence and risk factors of gastrointestinal bleeding in patients on low-dose aspirin therapy after percutaneous coronary intervention in Japan. *Scand J Gastroenterol* 2013; **48**: 320-325 [PMID: 23298342 DOI: 10.3109/00365521.2012.758771]

17 **Svoboda P**, Kantorova I, Scheer P, Doubek J, Ochmann J, Rehorkova D, Bosakova H. Helicobacter pylori is not a risk factor for stress ulcer bleeding in polytraumatized patients. *Hepatogastroenterology* 2004; **51**: 476-480 [PMID: 15086186]

18 **Aoyama N**, Kinoshita Y, Fujimoto S, Himeno S, Todo A, Kasuga M, Chiba T. Peptic ulcers after the Hanshin-Awaji earthquake: increased incidence of bleeding gastric ulcers. *Am J Gastroenterol* 1998; **93**: 311-316 [PMID: 9517630 DOI: 10.1111/j.1572-0241.1998.00311.x]

19 **Kurokawa M**, Nukina M, Nakanishi H, Miki K, Tomita S, Tohdo A. [The relationship between hemorrhagic gastric ulcers often appearing among in the great Hanshin-Awaji earthquake sufferers and Helicobacter pylori infections]. *Kansenshogaku Zasshi* 1996; **70**: 970-975 [PMID: 8921681]

20 **Matsushima Y**, Aoyama N, Fukuda H, Kinoshita Y, Todo A, Himeno S, Fujimoto S, Kasuga M, Nakase H, Chiba T. Gastric ulcer formation after the Hanshin-Awaji earthquake: a case study of Helicobacter pylori infection and stress-induced gastric ulcers. *Helicobacter* 1999; **4**: 94-99 [PMID: 10382122 DOI: 10.1046/j.1523-5378.1999.98290.x]

21 **Matsuura M**. [Series: Physicians and disaster medical care; The changes in gastrointestinal bleeding and the countermeasures at a major hospital after the Great East Japan Earthquake and the tsunami. Report from the hospital located in the disaster area]. *Nihon Naika Gakkai Zasshi* 2012; **101**: 2370-2374 [PMID: 22973716 DOI: 10.2169/naika.101.2370]

22 **Takakura R**, Himeno S, Kanayama Y, Sonoda T, Kiriyama K, Furubayashi T, Yabu M, Yoshida S, Nagasawa Y, Inoue S, Iwao N. Follow-up after the Hanshin-Awaji earthquake: diverse influences on pneumonia, bronchial asthma, peptic ulcer and diabetes mellitus. *Intern Med* 1997; **36**: 87-91 [PMID: 9099588 DOI: 10.2169/internalmedicine.36.87]

23 **Arisawa T**, Tahara T, Shibata T, Nagasaka M, Nakamura M, Kamiya Y, Fujita H, Nakamura M, Yoshioka D, Arima Y, Okubo M, Hirata I, Nakano H. Association between genetic polymorphisms in the cyclooxygenase-1 gene promoter and peptic ulcers in Japan. *Int J Mol Med* 2007; **20**: 373-378 [PMID: 17671743]

24 **Kanno T**, Iijima K, Abe Y, Koike T, Shimada N, Hoshi T, Sano N, Ohyauchi M, Ito H, Atsumi T, Konishi H, Asonuma S, Shimosegawa T. Hemorrhagic ulcers after Great East Japan Earthquake and Tsunami: features of post-disaster hemorrhagic ulcers. *Digestion* 2013; **87**: 40-46 [PMID: 23343968 DOI: 10.1159/000343937]

25 **Aoyama N**, Shinoda Y, Matsushima Y, Shirasaka D, Kinoshita Y, Kasuga M, Chiba T. Helicobacter pylori-negative peptic ulcer in Japan: which contributes most to peptic ulcer development, Helicobacter pylori, NSAIDS or stress? *J Gastroenterol* 2000; **35** Suppl 12: 33-37 [PMID: 10779215]

26 **Nishikawa K**, Sugiyama T, Kato M, Ishizuka J, Komatsu Y, Kagaya H, Katagiri M, Nishikawa S, Hokari K, Takeda H, Asaka M. Non-Helicobacter pylori and non-NSAID peptic ulcer disease in the Japanese population. *Eur J Gastroenterol Hepatol* 2000; **12**: 635-640 [PMID: 10912481 DOI: 10.1097/00042737-200012060-00010]

P- Reviewers: Ang D, Figura N, Kim BW, Matsuhashi N
S- Editor: Wen LL **L- Editor**: O'Neill M **E- Editor:** Liu XM

WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7433

World J Gastroenterol 2013 November 14; 19(42): 7433-7439
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Differentiation between dysplastic nodule and early-stage hepatocellular carcinoma: The utility of conventional MR imaging

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Author contributions: Chou CT and Chen RC designed the research; Chou CT, Chen CB, Chou JM, Chang TA, and Huang SF performed the research; Chen RC and Chen YL analyzed the data; and Chou CT, Chou JM and Chen RC wrote the manuscript.

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Received: June 22, 2013 **Revised:** August 16, 2013
Accepted: September 16, 2013
Published online: November 14, 2013

Abstract

AIM: To elucidate the variety of ways early-stage hepatocellular carcinoma (HCC) can appear on magnetic resonance (MR) imaging by analyzing T1-weighted, T2-weighted, and gadolinium-enhanced dynamic studies.

METHODS: Seventy-three patients with well-differentiated HCC (wHCC) or dysplastic nodules were retrospectively identified from medical records, and new histological sections were prepared and reviewed. The tumor nodules were categorized into three groups: dysplastic nodule (DN), wHCC compatible with Edmondson-Steiner grade Ⅰ HCC (w1-HCC), and wHCC compatible with Edmondson-Steiner grade Ⅱ HCC (w2-HCC). The signal intensity on pre-contrast MR imaging and the enhancing pattern for each tumor were recorded and compared between the three tumor groups.

RESULTS: Among the 73 patients, 14 were diagnosed as having DN, 40 were diagnosed as having w1-HCC, and 19 were diagnosed as having w2-HCC. Hyperintensity measurements on T2-weighted axial images (T2WI) were statistically significant between DNs and wHCC (P = 0.006) and between DN and w1-HCC (P = 0.02). The other imaging features revealed no significant differences between DN and wHCC or between DN and w1-HCC. Hyperintensity on both T1W out-phase imaging (P = 0.007) and arterial enhancement on dynamic study (P = 0.005) showed statistically significant differences between w1-HCC and w2-HCC. The other imaging features revealed no significant differences between w1-HCC and w2-HCC.

CONCLUSION: In the follow-up for a cirrhotic nodule, increased signal intensity on T2WI may be a sign of malignant transformation. Furthermore, a noted loss of hyperintensity on T1WI and the detection of arterial enhancement might indicate further progression of the histological grade.

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Key words: Dysplastic nodule; Hepatocellular carcinoma; Histological grading; Magnetic resonance imaging; Well-differentiated hepatocellular carcinoma

Core tip: The aim of this article was to differentiate between early-stage hepatocellular carcinoma (HCC) and dysplastic nodules using conventional magnetic resonance (MR) imaging. We found that conventional MR imaging could provide additional information to differentiate between early-stage HCC and dysplastic nodules in equivocal lesions. During follow-up for a cirrhotic nodules, increased signal intensity on T2-weighted axial images may be a sign of malignant transformation. Loss of hyperintensity on T1WI and the detection of arterial enhancement may indicate further progression of the histological grade.

Chou CT, Chou JM, Chang TA, Huang SF, Chen CB, Chen YL, Chen RC. Differentiation between dysplastic nodule and early-stage hepatocellular carcinoma: The utility of conventional MR imaging. *World J Gastroenterol* 2013; 19(42): 7433-7439 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7433.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7433

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most commonly diagnosed malignant tumors in the world. HCC occurs primarily in patients with chronic liver disease such as hepatitis B and C infections[1,2]. HCC develops by means of a multi-step dedifferentiation process that progresses from regenerative nodule to dysplastic nodule (DN) and then to HCC[3]. Early detection of HCC in cirrhotic livers is important to improve patient outcomes and decision-making to determine optimum therapeutic strategies[4]. A follow-up system for high-risk HCC populations has been established. Additionally, research and technology have enabled increasing numbers of small nodular lesions to be detected. According to the practice guidelines of the American Association for the Study of Liver Diseases (AASLD), HCC can be diagnosed noninvasively in at-risk patients, who typically demonstrate arterial-phase enhancement and venous- or delayed-phase washout on dynamic computed tomography (CT) or magnetic resonance imaging (MRI)[5]. However, most of these nodules with characteristic CT or MR patterns are overt HCC. The percentage of well-differentiated HCCs showing typical hypervascularity in the arterial-phase followed by washout in the delayed-phase on a dynamic study ranges from 13% to 50%[6,7]. Therefore, well-differentiated HCCs are increasingly detected in our daily practice, yet many represent diagnostic difficulties.

Gadoxetic acid (Gd-EOB-DTPA)-enhanced MRI has been demonstrated to be useful in differentiating between early HCC and dysplastic nodules in several recent studies[8,9]. However, the overlap between dysplastic nodules and HCC on gadoxetic acid-enhanced MR presentations has also been mentioned in the literature[10,11]. Due to the facts that gadoxetic acid is not available in every country and is costly, conventional MRI remains an important tool in differentiating between cirrhotic nodules and early HCC. Conventional MR imaging protocols rely on T1-weighted and T2-weighted imaging and multiphase dynamic gadolinium-enhanced imaging to depict and characterize tumors[12,13]. To the best of our knowledge, the role of conventional MR sequences in differentiating between early-stage HCC and dysplastic nodule in cirrhotic liver has not yet been established. The purpose of our study was to elucidate the variety ways of early-stage HCC can appear on MR imaging by analyzing T1-weighted, T2-weighted, and gadolinium-enhanced dynamic studies.

MATERIALS AND METHODS

Patient population

Approval for this retrospective study was obtained from the institutional review board of our hospital. A flowchart for the patient selection process is shown in Figure 1. Ultimately, 73 patients (51 men and 22 women; mean age, 61 years old; range, 26-82 years) were enrolled in this study. Among them, 70 patients underwent liver biopsy, and 3 patients underwent hepatectomy. All 73 patients had liver cirrhosis: 29 patients had hepatitis B virus; 28 patients had hepatitis C virus; 9 patients had hepatitis B and C co-infections; and 7 patients had alcoholic cirrhosis. Among the 73 patients, 49 patients had Child-Pugh class A cirrhosis, and 24 patients had Child-Pugh class B cirrhosis. The diagnoses of liver cirrhosis were all based on histopathological examination.

Histopathological diagnosis

New histological sections from the original paraffin blocks of all 73 patients' liver tumors were prepared using hematoxylin and eosin stain. The diagnostic features for HCC included the following: (1) increased cell density >2 times the density of the surrounding tissue, with an increased nuclear:cytoplasmic ratio; (2) thickened liver cell plates (2 cells or more); (3) pseudoglandular structure formations; (4) unpaired arteries; (5) sinusoidal capillarization; and (6) stromal invasion without ductular reaction at the periphery of the nodules[14,15]. Immunohistochemical stains for CD10 (Biocare Medical, Concord, CA, United States), CD34 (DAKO, Glostrup, Denmark) and cytokeratin 7 (DAKO, Glostrup, Denmark) were also performed to facilitate interpretation. The tumor nodules were categorized into three groups: DN, well-differentiated HCC compatible with Edmondson-Steiner grade Ⅰ HCC (w1-HCC)[16], and well-differentiated HCC compatible with Edmondson-Steiner grade Ⅱ HCC [which also included well-differentiated HCC in the World Health Organization system (w2-HCC)[17].

Two pathologists with more than 10 years of experience in hepatic pathology reviewed all the histological sections independently. Differences between the two reviewers were resolved by a third pathologist to reach a final consensus. All three of the pathologists were blinded to the original pathology diagnoses and the clinical information.

The presence of high cellularity, diffuse capillarization, abnormal biliary canaliculi and stromal invasion were considered features of w1-HCC. The additional presence of nuclear pleomorphism and thicker trabecular growth further upgraded a lesion to, w2-HCC.

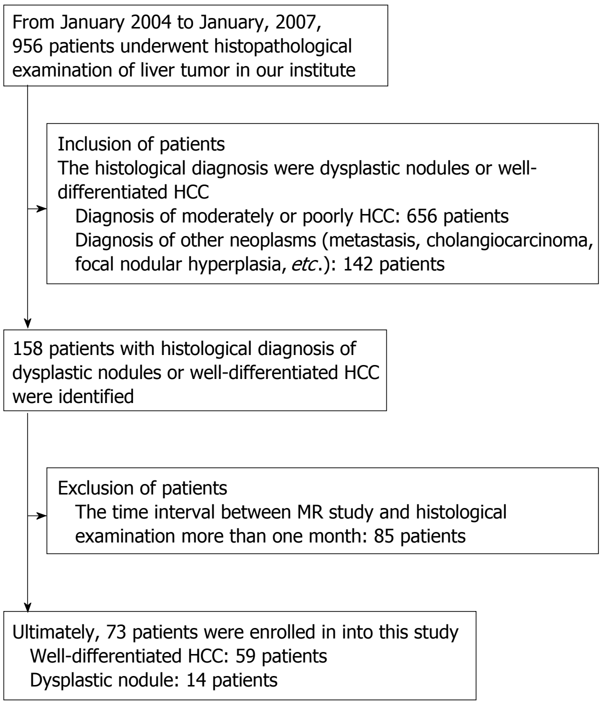
From January 2004 to January, 2007,
956 patients underwent histopathological
examination of liver tumor in our institute
Inclusion of patients
The histological diagnosis were dysplastic nodules or well-differentiated HCC
Diagnosis of moderately or poorly HCC: 656 patients
Diagnosis of other neoplasms (metastasis, cholangiocarcinoma, focal nodular hyperplasia, *etc*.): 142 patients
158 patients with histological diagnosis of dysplastic nodules or well-differentiated HCC were identified
Exclusion of patients
The time interval between MR study and histological examination more than one month: 85 patients
Ultimately, 73 patients were enrolled in into this study
Well-differentiated HCC: 59 patients
Dysplastic nodule: 14 patients

Figure 1 Flowchart of the patient selection process. HCC: Hepatocellular carcinoma; MR: Magnetic resonance.

MR imaging

MR imaging of livers was performed using a 1.5-T MR scanner (Philips Gyroscan ACS-NT Powertrak 6000, release version 6.7.2, Best, the Netherlands) and a phased-array body coil. Turbo spin-echo (TSE) T2-weighted axial images [T2WI, TR/TE: 2500/90 ms, slice thickness/gap: 8/0.8 mm, matrix: 192 × 256, number of average (NEX): 2, TSE factor: 23, field of view (FOV): 38-40 cm, typical scanning time: 2 min 20 s] with and without fat saturation (FS, spectral fat saturation inversion recovery) and coronal T2-weighted images were obtained under respiratory trigger. Dual-echo T1-weighted imaging (TR/TE: 210/2.3 ms and 4.6 ms, slice thickness/gap: 8/0.8 mm, matrix: 192 × 256, NEX: 1, FOV: 38-40 cm, typical scanning time: 24 s) was also performed during one breath hold. Automatic shimming was applied for fat-suppression imaging to maximize magnetic field homogeneity, and flow compensation was also used.

For contrast-enhanced MR imaging, gadodiamide (Omniscan, GE Healthcare, Oslo, Norway) was administered by bolus injection (approximate rate of 2 mL/s) through a peripheral vein at a dosage of 0.1 mmol/kg. Dynamic T1-weighted fast field echo imaging (175-210/1.3-2.1, flip angle: 80°, matrix: 192 × 256, NEX: 1, FOV: 38-40 cm) was performed just before, 18-20 s after and 50-55 s after the contrast agent was injected. An equilibrium phase FS-T1W (TR/TE 241-344/2.7 ms, flip angle: 70°, slices thickness/gap: 8/0.8 mm) imaging was performed 180 s after the contrast agent injection.

Imaging analysis

The imaging analysis was performed at a dual-screen diagnostic workstation (GE Healthcare, Milwaukee, WI, United States). In each image assessment, liver maps were completed by drawing each individual liver lesion on a respective map according to the Couinaud system of liver anatomy. These drawings were made as accurately as possible by one investigator. All the imaging results were analyzed using visual assessment by two radiologists who each had more than 10 years of experience in abdominal MR imaging. The two observers were blinded to the clinical information and final diagnoses, and they recorded the lesion signal intensities on pre-contrast T1WI and T2WI, post-contrast T1WI and the enhancement pattern during dynamic study. The signal intensity of the focal liver nodule on dual-echo T1WI and T2WI was classified as hypointense, isointense, or hyperintense compared with adjacent liver parenchyma. The enhancement pattern of the HCC was visually classified into one of the following patterns: hypovascular, isovascular or hypervascular enhancement compared with the adjacent liver parenchyma. Any disagreements between the two reviewers were resolved by consensus with a third radiologist who was also blinded to the clinical information and final diagnoses.

Statistical analysis

The interobserver agreement was evaluated using the kappa statistic[18]. Continuous variables such as age, tumor size, and alpha-fetoprotein were analyzed using the Kruskal-Wallis test. Categorical variables such as signal intensity and enhancement pattern were analyzed using Pearson's χ^2 test. The diagnostic performance of the HCC diagnostic criteria was evaluated along with a receiver operating characteristic analysis. A P value less than 0.05 was considered statistically significant.

RESULTS

Among the 73 patients in our study, 14 were diagnosed with a DN, 40 were diagnosed with w1-HCC, and 19 were diagnosed with w2-HCC. The clinicopathological characteristics of the growths are shown in Table 1, which shows no significant differences between the three patient groups. The interobserver agreement for the imaging analysis between the two radiologists was either good or excellent (Table 2).

Univariate analyses of MR findings for patients with DNs and wHCC are shown in Table 3. Only a hyperintense signal on T2WI was a statistically significant predictor of wHCC (P = 0.006), whereas the other imaging features revealed no significant associations with DN and wHCC. Therefore, well-differentiated HCCs were divided into w1-HCC and w2-HCC, and the differences between DN *vs* w1-HCC and w1-HCC *vs* w2-HCC were analyzed. Only hyperintensity on T2WI was a statistically significant differentiator of DN from w1-HCC (P = 0.02). The other imaging features revealed no significant differences between DN and w1-HCC. Hyperintensity on T1W out-phase imaging (P = 0.007) and arterial enhancement on dynamic study (P = 0.005) showed statistically significant

Table 1 Clinical characteristics of the 73 cirrhotic patients with liver nodules

	Histopathological diagnosis			P value
	DN ($n = 14$)	w1-HCC ($n = 40$)	w2-HCC ($n = 19$)	
Age (mean ± SD, yr)	54.9 ± 12.9	62.4 ± 12.2	63.4 ± 10.8	0.129
Sex				0.629
Male	11	27	12	
Female	3	13	7	
Underlying liver disease				0.640
HBV	7	15	7	
HCV	3	15	10	
HBV + HCV	2	6	1	
Alcoholism	2	4	1	
Child-Pugh class				0.645
A	10	25	14	
B	4	15	5	
Tumor size (mean ± SD, cm)	1.8 ± 0.6	2.1 ± 0.7	2.9 ± 2.3	0.365
AFP (mean ± SD, ng/mL)	17.1 ± 27.9	55.5 ± 117.9	64.2 ± 169.7	0.202
< 20 ng/mL	12	28	13	0.790
⩾ 20 ng/mL	2	12	6	
> 20, ⩽ 100 ng/mL	2	7	4	
> 100, ⩽ 200 ng/mL	0	0	1	
> 200 ng/mL	0	5	1	

HBV: Hepatitis B virus; HCV: Hepatitis C virus; AFP: Alpha-fetal protein; DN: Dysplastic nodule; w1-HCC: Well-differentiated hepatocellular carcinoma compatible with Edmondson-Steiner grade Ⅰ; w2-HCC Well-differentiated hepatocellular carcinoma compatible with Edmondson-Steiner grade Ⅱ.

differences between w1-HCC and w2-HCC. However, T2WI and other imaging sequences showed no significant differences between w1-HCC and w2-HCC.

Using hyperintensity on T2WI as the sole criteria in differentiating wHCC from a dysplastic nodule, 25 of 59 wHCCs could be correctly diagnosed. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 42%, 93%, 96% and 28%, respectively. Solely using the AASLD criteria (arterial enhancement followed by washout on dynamic imaging) to differentiate between wHCC and dysplastic nodules, only 9 of 59 wHCCs (w1-HCC: 2, w2-HCC: 7) were correctly diagnosed. The sensitivity, specificity, PPV and NPV were 15%, 93%, 90% and 21%, respectively. If the diagnostic criteria were changed to either a hyperintense nodule on T2WI or a lesion that demonstrated arterial enhancement followed by washout on dynamic imaging, 27 of 59 wHCCs were correctly diagnosed. The sensitivity, specificity, PPV, and NPV were 46%, 86%, 93%, and 27%, respectively. Eighteen wHCCs were further detected by the additional hyperintense-on-T2WI criterion according to the AASLD criteria. The diagnostic performance of the AASLD criteria with additional hyperintense findings on T2WI was superior to the AASLD criteria alone in the characterization of early-stage HCC ($P = 0.013$).

Table 2 Interobserver agreement for magnetic resonance features

MR features	*k* value
T1-weighted in-phase imaging	0.853
T1-weighted opposed-phase imaging	0.870
Fatty metamorphosis	0.819
T2-weighted imaging	0.828
Arterial enhancement	0.806
Late-phase T1-weighted imaging	0.743

MR: Magnetic resonance.

DISCUSSION

The development of HCC in the cirrhotic liver is described as either *de novo* hepatocarcinogenesis or as the result of a multistep progression. The stages of the multistep progression originate from a dysplastic nodule, which progresses to a dysplastic nodule with HCC foci, followed by a small HCC, and finally to an overt carcinoma[19,20]. In our study, typical arterial enhancing features were only detected in 12 (12/40) w1-HCCs, and there were no significant differences between DNs and w1-HCC. In contrast, the arterial enhancing feature was demonstrated in 13 (13/19) w2-HCCs and showed a statistically significant difference between w1-HCC and w2-HCC ($P = 0.005$). Our results might explain why the percentage of well-differentiated HCCs with arterial enhancement ranges from 43% to 66% in the current literature[21-23]. These figures may have resulted from the varied compositions of w1-HCC and w2-HCC within the studies.

The arterial enhancement during dynamic study showed a statistically significant difference between w1-HCC and w2-HCC in our study. This finding might be due to the insufficient development of non-triadal arteries in early-stage HCCs[14,24]. Kojiro[25] also reported that early HCCs tended to demonstrate histological hypovascularity. Kim *et al*[26] reported that the histological grade of HCC was an important factor influencing therapeutic results. They further suggested that treatment could be more effective after radiofrequency ablation in patients with histologically low-grade HCC. According to our results, wHCC demonstrated arterial enhancement during dynamic study, and the histological differentiation tended to be of a higher grade, so it should be treated aggressively.

In our results, only 9 wHCCs (15%) satisfied the AASLD HCC diagnostic criteria. In contrast, T2WI hyperintensity was the only imaging feature that allowed differentiation between wHCC and DN or w1-HCC and DN. The diagnostic performance of the AASLD criteria with additional hyperintensity on T2WI was superior to the AASLD criteria alone in the characterization of early-stage HCCs ($P = 0.013$). Ouedraogo *et al*[27] also reported that adding T2W hyperintensity to the AASLD criteria increased the detection of HCC, especially in nodules smaller than 20 mm. According to our results, hyperintensity on T2WI alone could offer additional information in dynamic studies to differentiate between wHCC and DNs, and a cirrhotic nodule that is hyperintense on

Table 3 Comparison of magnetic resonance features between dysplastic nodule and well-differentiated hepatocellular carcinoma

	DN (n = 14)	wHCC		*P* value		
		w1 (n = 40)	w2 (n = 19)	DN *vs* wHCC	DN *vs* w1	w1 *vs* w2
In-phase T1WI				0.469	0.405	0.074
Hyperintense	8	29	8			
Isointense	4	5	4			
Hypointense	2	6	7			
Opposed-phase T1WI				0.661	0.565	0.007
Hyperintense	8	25	4			
Isointense	3	4	6			
Hypointense	3	11	9			
Fatty metamorphosis				0.192	0.311	0.450
Positive	0	5	4			
Negative	14	35	15			
T2WI				0.006	0.020	0.109
Hyperintense	1	14	12			
Isointense	6	5	2			
Hypointense	7	21	5			
Arterial enhancement				0.767	0.753	0.005
Hypervascular	5	12	13			
Iso-/hypo-vascular	9	28	6			
Late-phase T1WI				0.112	0.286	0.105
Hyperintense	1	5	3			
Isointense	12	26	7			
Hypointense	1	9	9			

T1WI: T1-weighted imaging; T2WI: T2-weighted imaging; DN: Dysplastic nodule; wHCC: Well-differentiated hepatocellular carcinoma; w1: wHCC compatible with Edmondson-Steiner grade Ⅰ; w2: wHCC compatible with Edmondson-Steiner grade Ⅱ.

T2WI should be aggressively biopsied.

In our study, hyperintensity on T1W out-phase imaging showed a significant difference between w1-HCC and w2-HCC. A hyperintense HCC on unenhanced T1W images tended to be lower grade histologically. This finding might be because borderline lesions and some early HCCs/wHCCs are occasionally hyperintense on unenhanced T1W images[28,29]. Matsui *et al*[30] correlated MR signal intensity with tumor histology, and considered cell crowding, fatty accumulation and possibly copper deposition to be responsible for the hyperintensity on T1W imaging. However, the exact histological composition responsible for the signal intensity characteristics of w1-HCC and w2-HCC remains elusive.

According to the step-wise carcinogenesis model, HCC changes in appearance through the course of its development. These changes include a steadily increasing signal intensity on T2WI with gradually increasing neovascularity in most lesions. Van den Bos *et al*[31] suggested that the increased signal on T2W images lags behind the developing neovascularity. Our results supported the proposition that an increased signal on T2W images occurs early in the developing arterial enhancement. These discrepancies in enhancement patterns and signal intensities might be due to the different patient populations being examined and the different underlying liver diseases. However, more studies are needed for a better understanding of developing HCC using MR imaging.

Recent progress in CT angiography, Gd-EOB-DTPA-enhanced MRI, diffusion-weighted imaging (DWI), contrast-enhanced ultrasound (CEUS) has made these modalities useful in differentiating HCC from dysplastic nodules. Lee *et al*[11] reported that hypointensity on Gd-EOB-DTPA-enhanced hepatobiliary-phase images and hyperintensity on high-b-value DWI in the surrounding liver parenchyma were useful in differentiating wHCC from benign nodules. Kudo[3] reported that CT during hepatic angiography and CT during arterial portography were the most sensitive tools in differentiating between premalignant/borderline lesions and early HCC. Real-time CEUS has the ability to detect slowly enhancing HCCs, which on CT could be interpreted as hypovascular lesions[32]. Giorgio *et al*[33] reported that DN, early HCC and progressed HCC could be accurately differentiated using CEUS on the basis of the vascularization pattern during the arterial phase. Kudo[34] reported that Sonazoid-enhanced US could generate both hemodynamic-phase and Kupper-phase images and offer improved diagnostic performance for focal liver lesions. CEUS with Sonazoid may play an important role in the characterization of focal hepatic lesions in the future.

This study had two main limitations. First, the study was retrospective. If we had used the original pathohistological reports in the medical records as a standard of reference, inaccuracies due to differing standards of pathological interpretation at that time might have led to different results. To ensure accurate histological diagnoses, new histological slides were prepared from paraffin-embedded blocks, all of which were read by two experienced pathologists independently. A third pathologist was involved as needed to reach a final consensus when disagreements arose. The second limitation was that the histological diagnoses of 70 of the nodules (70/73) were based on needle biopsies. Potential sampling errors and sampling variation are inherent in this type of examination, and they are recognized shortcomings in most comparative studies.

In conclusion, conventional MR imaging could provide additional information to differentiate between wHCC and DN in equivocal lesions. The variable presentation of wHCC in the current literature may be due to the differing cellular compositions of w1-HCC and w2-HCC. Consequently, during follow-up of a cirrhotic nodule, increased signal intensity on T2WI may be a sign of malignant transformation. Loss of hyperintensity in T1WI and the detection of arterial enhancement may indicate further progression of the histological grade.

COMMENTS

Background

Most well-differentiated hepatocellular carcinomas (HCCs) at an early stage do not demonstrate hypervascularity upon dynamic computed tomography/magnetic resonance (CT/MR) study, thereby making their diagnosis difficult. Some well-differentiated HCCs are fed by the portal vein instead of the hepatic artery and may look like benign nodules with "benign-appearing" patterns of vasculature. Gadoxetic acid-enhanced magnetic resonance imaging (MRI) and contrast-enhanced ultrasound have been demonstrated to be useful in differentiating between early-stage HCC and dysplastic nodules in several recent studies. Due to the facts that gadoxetic acid and contrast medium for ultrasound are not available in every country and are costly, conventional MRI remains an important tool in differentiating between cirrhotic nodules and early HCCs. The authors used a retrospective analysis to show the utility of MR imaging features to differentiate between dysplastic nodules and early stage HCC.

Research frontiers

The authors compared conventional MR imaging results with histological results to evaluate the effectiveness of conventional MR imaging in the diagnosis of early-stage HCC.

Innovations and breakthroughs

Increased signal intensity on T2-weighted axial images (T2WI) may be a sign of malignant transformation. Loss of hyperintensity on T1-weighted axial images (T1W1) and the detection of arterial enhancement may indicate further progression of the histological grade.

Applications

The authors believe that conventional MR imaging could provide additional information in differentiating between early-stage HCC and dysplastic nodule in equivocal lesions. During follow-up for a cirrhotic nodule, increased signal intensity on T2WI may be a sign of malignant transformation and should be biopsied. Loss of hyperintensity on T1WI and the detection of arterial enhancement may indicate further progression of the histological grade and should be treated aggressively.

Peer review

The authors describe a retrospective analysis of 73 patients with well-differentiated HCC or dysplastic nodules. The authors address the question whether MR imaging is helpful to differentiate between a dysplastic nodules and well-differentiated HCC in high-risk patients with cirrhosis. The question addressed by the authors is of high impact in the field of liver cirrhosis. Despite the limitations of the study (retrospective analysis, 73 patients), the authors have thoroughly analyzed the data and have made new histological classifications of all the specimens. The data show that increased signal intensity on T2WI may be a sign of malignant transformation.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

2 **Donato F**, Tagger A, Chiesa R, Ribero ML, Tomasoni V, Fasola M, Gelatti U, Portera G, Boffetta P, Nardi G. Hepatitis B and C virus infection, alcohol drinking, and hepatocellular carcinoma: a case-control study in Italy. Brescia HCC Study. *Hepatology* 1997; **26**: 579-584 [PMID: 9303486 DOI: 10.1002/hep.510260308]

3 **Kudo M**. Multistep human hepatocarcinogenesis: correlation of imaging with pathology. *J Gastroenterol* 2009; **44** Suppl 19: 112-118 [PMID: 19148804 DOI: 10.1007/s00535-008-2274-6]

4 **Szklaruk J**, Silverman PM, Charnsangavej C. Imaging in the diagnosis, staging, treatment, and surveillance of hepatocellular carcinoma. *AJR Am J Roentgenol* 2003; **180**: 441-454 [PMID: 12540450 DOI: 10.2214/ajr.180.2.1800441]

5 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

6 **Yoon SH**, Lee JM, So YH, Hong SH, Kim SJ, Han JK, Choi BI. Multiphasic MDCT enhancement pattern of hepatocellular carcinoma smaller than 3 cm in diameter: tumor size and cellular differentiation. *AJR Am J Roentgenol* 2009; **193**: W482-W489 [PMID: 19933622 DOI: 10.2214/AJR.08.1818]

7 **Li CS**, Chen RC, Tu HY, Shih LS, Zhang TA, Lii JM, Chen WT, Duh SJ, Chiang LC. Imaging well-differentiated hepatocellular carcinoma with dynamic triple-phase helical computed tomography. *Br J Radiol* 2006; **79**: 659-665 [PMID: 16641423 DOI: 10.1259/bjr/12699987]

8 **Kudo M**. The 2008 Okuda lecture: Management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. *J Gastroenterol Hepatol* 2010; **25**: 439-452 [PMID: 20370723 DOI: 10.1111/j.1440-1746.2009.06207.x]

9 **Okada M**, Imai Y, Kim T, Kogita S, Takamura M, Kumano S, Onishi H, Hori M, Fukuda K, Hayashi N, Wakasa K, Sakamoto M, Murakami T. Comparison of enhancement patterns of histologically confirmed hepatocellular carcinoma between gadoxetate- and ferucarbotran-enhanced magnetic resonance imaging. *J Magn Reson Imaging* 2010; **32**: 903-913 [PMID: 20882621 DOI: 10.1002/jmri.22333]

10 **Kogita S**, Imai Y, Okada M, Kim T, Onishi H, Takamura M, Fukuda K, Igura T, Sawai Y, Morimoto O, Hori M, Nagano H, Wakasa K, Hayashi N, Murakami T. Gd-EOB-DTPA-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading and portal blood flow. *Eur Radiol* 2010; **20**: 2405-2413 [PMID: 20490505 DOI: 10.1007/s00330-010-1812-9]

11 **Lee MH**, Kim SH, Park MJ, Park CK, Rhim H. Gadoxetic acid-enhanced hepatobiliary phase MRI and high-b-value diffusion-weighted imaging to distinguish well-differentiated hepatocellular carcinomas from benign nodules in patients with chronic liver disease. *AJR Am J Roentgenol* 2011; **197**: W868-W875 [PMID: 22021534 DOI: 10.2214/AJR.10.6237]

12 **Lee JM**, Choi BI. Hepatocellular nodules in liver cirrhosis: MR evaluation. *Abdom Imaging* 2011; **36**: 282-289 [PMID: 21399975 DOI: 10.1007/s00261-011-9692-2]

13 **Willatt JM**, Hussain HK, Adusumilli S, Marrero JA. MR Imaging of hepatocellular carcinoma in the cirrhotic liver: challenges and controversies. *Radiology* 2008; **247**: 311-330 [PMID: 18430871 DOI: 10.1148/radiol.2472061331]

14 **Roskams T**, Kojiro M. Pathology of early hepatocellular carcinoma: conventional and molecular diagnosis. *Semin Liver Dis* 2010; **30**: 17-25 [PMID: 20175030 DOI: 10.1055/s-0030-1247129]

15 **Hytiroglou P**, Park YN, Krinsky G, Theise ND. Hepatic precancerous lesions and small hepatocellular carcinoma. *Gastroenterol Clin North Am* 2007; **36**: 867-87, vii [PMID: 17996795 DOI: 10.1016/j.gtc.2007.08.010]

16 **Edmondson HA**, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 1954; **7**: 462-503 [PMID: 13160935 DOI: 10.1002/1097-0142(195405)7]

17 **Hamilton SR**, Aaltonen LA. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Digestive System. Lyon, France: IARC Press, 2000: 159-172

18 **Landis JR**, Koch GG. The measurement of observer agree-

ment for categorical data. *Biometrics* 1977; **33**: 159-174 [PMID: 843571 DOI: 10.2307/2529310]

19 **Coleman WB**. Mechanisms of human hepatocarcinogenesis. *Curr Mol Med* 2003; **3**: 573-588 [PMID: 14527088 DOI: 10.2174/1566524033479546]

20 **Efremidis SC**, Hytiroglou P. The multistep process of hepatocarcinogenesis in cirrhosis with imaging correlation. *Eur Radiol* 2002; **12**: 753-764 [PMID: 11960222 DOI: 10.1007/s00330-001-1142-z]

21 **Jang HJ**, Kim TK, Burns PN, Wilson SR. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. *Radiology* 2007; **244**: 898-906 [PMID: 17709836 DOI: 10.1148/radiol.2443061520]

22 **Saitoh S**, Ikeda K, Koida I, Tsubota A, Arase Y, Chayama K, Kumada H. Serial hemodynamic measurements in well-differentiated hepatocellular carcinomas. *Hepatology* 1995; **21**: 1530-1534 [PMID: 7768496 DOI: 10.1016/0270-9139(95)90455-7]

23 **Arita J**, Hasegawa K, Takahashi M, Hata S, Shindoh J, Sugawara Y, Kokudo N. Correlation between contrast-enhanced intraoperative ultrasound using Sonazoid and histologic grade of resected hepatocellular carcinoma. *AJR Am J Roentgenol* 2011; **196**: 1314-1321 [PMID: 21606294 DOI: 10.2214/AJR.10.4310]

24 **Nakashima Y**, Nakashima O, Hsia CC, Kojiro M, Tabor E. Vascularization of small hepatocellular carcinomas: correlation with differentiation. *Liver* 1999; **19**: 12-18 [PMID: 9928760 DOI: 10.1111/j.1478-3231.1999.tb00003.x]

25 **Kojiro M**. Focus on dysplastic nodules and early hepatocellular carcinoma: an Eastern point of view. *Liver Transpl* 2004; **10**: S3-S8 [PMID: 14762831 DOI: 10.1002/lt.20042]

26 **Kim SH**, Lim HK, Choi D, Lee WJ, Kim SH, Kim MJ, Kim CK, Jeon YH, Lee JM, Rhim H. Percutaneous radiofrequency ablation of hepatocellular carcinoma: effect of histologic grade on therapeutic results. *AJR Am J Roentgenol* 2006; **186**: S327-S333 [PMID: 16632696 DOI: 10.2214/AJR.05.0350]

27 **Ouedraogo W**, Tran-Van Nhieu J, Baranes L, Lin SJ, Decaens T, Laurent A, Djabbari M, Pigneur F, Duvoux C, Kobeiter H, Deux JF, Rahmouni A, Luciani A. [Evaluation of noninvasive diagnostic criteria for hepatocellular carcinoma on pretransplant MRI (2010): correlation between MR imaging features and histological features on liver specimen]. *J Radiol* 2011; **92**: 688-700 [PMID: 21819911 DOI: 10.1016/j.jradio.2011.03.020]

28 **Shinmura R**, Matsui O, Kobayashi S, Terayama N, Sanada J, Ueda K, Gabata T, Kadoya M, Miyayama S. Cirrhotic nodules: association between MR imaging signal intensity and intranodular blood supply. *Radiology* 2005; **237**: 512-519 [PMID: 16244260 DOI: 10.1148/radiol.2372041389]

29 **Efremidis SC**, Hytiroglou P, Matsui O. Enhancement patterns and signal-intensity characteristics of small hepatocellular carcinoma in cirrhosis: pathologic basis and diagnostic challenges. *Eur Radiol* 2007; **17**: 2969-2982 [PMID: 17618439 DOI: 10.1007/s00330-007-0705-z]

30 **Matsui O**, Kadoya M, Kameyama T, Yoshikawa J, Arai K, Gabata T, Takashima T, Nakanuma Y, Terada T, Ida M. Adenomatous hyperplastic nodules in the cirrhotic liver: differentiation from hepatocellular carcinoma with MR imaging. *Radiology* 1989; **173**: 123-126 [PMID: 2550995]

31 **van den Bos IC**, Hussain SM, Terkivatan T, Zondervan PE, de Man RA. Stepwise carcinogenesis of hepatocellular carcinoma in the cirrhotic liver: demonstration on serial MR imaging. *J Magn Reson Imaging* 2006; **24**: 1071-1080 [PMID: 17024654 DOI: 10.1002/jmri.20701]

32 **Nicolau C**, Vilana R, Bianchi L, Brú C. Early-stage hepatocellular carcinoma: the high accuracy of real-time contrast-enhanced ultrasonography in the assessment of response to percutaneous treatment. *Eur Radiol* 2007; **17** Suppl 6: F80-F88 [PMID: 18376461]

33 **Giorgio A**, Calisti G, di Sarno A, Farella N, de Stefano G, Scognamiglio U, Giorgio V. Characterization of dysplastic nodules, early hepatocellular carcinoma and progressed hepatocellular carcinoma in cirrhosis with contrast-enhanced ultrasound. *Anticancer Res* 2011; **31**: 3977-3982 [PMID: 22110230]

34 **Kudo M**. Diagnostic imaging of hepatocellular carcinoma: recent progress. *Oncology* 2011; **81** Suppl 1: 73-85 [PMID: 22212940 DOI: 10.1159/000333265]

P- Reviewers: Enomoto N, Hinz S, Singal AK **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Zhang DN

WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7440

World J Gastroenterol 2013 November 14; 19(42): 7440-7446
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Curcumin protects against acetaminophen-induced apoptosis in hepatic injury

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Author contributions: Li G and Chen JB performed the experiments, collected the data and wrote the manuscript; Wang C and Nie H designed the study and revised the manuscript; Xu Z, Qin XY and Chen XM performed the experiments and analyzed the data; Gong Q revised the manuscript.

Supported by National Natural Science Foundation of China, No. 81271872; Health Department of Hubei Province, No. XF2012-5; and Jingzhou Bureau of Science and Technology

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Received: June 5, 2013 **Revised:** August 26, 2013
Accepted: September 15, 2013
Published online: November 14, 2013

Abstract

AIM: To explore the effects of curcumin (CMN) on hepatic injury induced by acetaminophen (APAP) *in vivo*.

METHODS: Male mice were randomly divided into three groups: group Ⅰ (control) mice received the equivalent volumes of phosphate-buffered saline (PBS) intraperitoneally (*ip*); Group Ⅱ [APAP + carboxymethylcellulose (CMC)] mice received 1% CMC (vehicle) 2 h before APAP injection; Group Ⅲ (APAP + CMN) mice received curcumin (10 or 20 mg/kg, *ip*) 2 h before before or after APAP challenge. In Groups Ⅱ and Ⅲ, APAP was dissolved in pyrogen-free PBS and injected at a single dose of 300 mg/kg. CMN was dissolved in 1% CMC. Mice were sacrificed 16 h after the APAP injection to determine alanine aminotransferase (ALT) levels in serum and malondialdehyde (MDA) accumulation, superoxide dismutase (SOD) activity and hepatocyte apoptosis in liver tissues.

RESULTS: Both pre- and post-treatment with curcumin resulted in a significant decrease in serum ALT compared with APAP treatment group (10 mg/kg: 801.46 ± 661.34 U/L; 20 mg/kg: 99.68 ± 86.48 U/L *vs* 5406.80 ± 1785.75 U/L, $P < 0.001$, respectively). The incidence of liver necrosis was significantly lowered in CMN treated animals. MDA contents were significantly reduced in 20 mg/kg CMN pretreatment group, but increased in APAP treated group (10.96 ± 0.87 nmol/mg protein *vs* 16.03 ± 2.58 nmol/mg protein, $P < 0.05$). The decrease of SOD activity in APAP treatment group and the increase of SOD in 20 mg/kg CMN pretreatment group were also detected (24.54 ± 4.95 U/mg protein *vs* 50.21 ± 1.93 U/mg protein, $P < 0.05$). Furthermore, CMN treatment efficiently protected against APAP-induced apoptosis *via* increasing Bcl-2/Bax ratio.

CONCLUSION: CMN has significant therapeutic potential in both APAP-induced hepatotoxicity and other types of liver diseases.

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Key words: Acetaminophen; Acute hepatic injury; Apoptosis; Free radicals; Curcumin

Core tip: Acetaminophen (APAP) and curcumin (CMN) were administrated intraperitoneally. The aim of the study was to explore whether CMN has effect on APAP-induced hepatic toxicity *in vivo*. The findings revealed that CMN protects against APAP-induced lipid peroxidation, oxidative stress and hepatocyte apoptosis.

Li G, Chen JB, Wang C, Xu Z, Nie H, Qin XY, Chen XM, Gong Q. Curcumin protects against acetaminophen-induced apoptosis in hepatic injury. *World J Gastroenterol* 2013; 19(42): 7440-7446 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7440.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7440

INTRODUCTION

Acetaminophen (APAP) is a widely used analgesic and antipyretic drug that is safe and effective when taken at therapeutic dose[1]. However, it can cause severe liver damage or even acute liver failure that can be fatal in experimental animals and humans when administered in an acute or cumulative overdose[2,3]. APAP overdose is the leading cause for calls to Poison Control Centers (> 100000/year) and accounts for more than 56000 emergency visits, 2600 hospitalizations, and an estimated 458 deaths each year in the United States[3].

APAP is metabolized by cytochrome P450 to *N*-acetyl-p-benzoquinone imine (NAPQI). NAPQI can react rapidly with glutathione (GSH), so large doses of APAP may result in a profound depletion of hepatocellular GSH[1,4]. Once GSH is exhausted, any remaining NAPQI will covalently bind to cellular proteins and induce mitochondrial dysfunction, lipid peroxidation, oxidative stress, and DNA fragmentation, eventually leads to massive hepatocyte necrosis, liver damage or death[5]. *N*-acetyl cysteine has been currently used in the treatment of APAP-induced liver toxicity[6]. In addition to its adverse reaction, a major concern when using *N*-acetyl cysteine is its relatively narrow therapeutic windows and drug toxicity[7,8]. Therefore, new and safe preventive measures against APAP toxicity are eagerly needed.

In recent years, natural products from plants have received considerable attention as a rich resource for drug development. Curcumin (CMN) is a yellow pigment purified from the root tubers of *Curcuma longa Linn* (commonly known as turmeric), which has long been used as a food colorant and preservative[9]. CMN also has a variety of biological and pharmacological activities, such as anti-inflammatory, anti-oxidant, antifungal, antibacterial and anticancer activities[10]. It was reported that CMN attenuates liver injury induced by ethanol[11], iron overdose[12] and carbon tetrachloride intoxication[13].

The aim of this study was to explore the effect of CMN on the prevention of APAP-induced hepatic toxicity *in vivo* and investigate whether CMN affects the production of lipid peroxidation, oxidative stress or hepatocyte apoptosis to attenuate liver damage.

MATERIALS AND METHODS

Materials

APAP and CMN were purchased from Sigma Aldrich (Saint Louis, MO, United States). Detection kits for superoxide dismutase (SOD) and malondial dehyde (MDA) were purchased from Nanjing Jiancheng Bioengineer Institute (Nanjing, China). Transferase-mediated dUTP-biotin nick end labeling (TUNEL) detection kit was purchased from Boster Biological Technology Co., Ltd (Wuhan, China).

Animals and treatment

Male BALB/c mice (6-8 wk of age) were purchased from the Center for Animal Experiment of Wuhan University (Wuhan, China). The mice were raised at an animal facility under special pathogen-free conditions with a 12-h light/dark cycle and free access to food and water at least 1 wk prior to treatment. All animal experiments were approved by the institutional animal care and use committee at the Yangtze University, and all efforts were made to minimize the number of animals used and their sufferings. Mice were randomly divided into three groups: group Ⅰ (control) mice received the equivalent volumes of PBS intraperitoneally (*ip*); Group Ⅱ (APAP + CMC) mice received 1% CMC (vehicle) 2 h before APAP injection; and Group Ⅲ (APAP + CMN) mice received CMN (10 or 20 mg/kg, *ip*) 2 h before or after APAP challenge. In Group Ⅱ and Group Ⅲ, APAP was dissolved in pyrogen-free PBS and injected at a single dose of 300 mg/kg; the dose was selected on the basis of a previous related study[14]. CMN was dissolved in 1% CMC. Serum and livers were collected at 16 h after APAP treatment.

Biochemical analysis

Alanine amino-transaminase (ALT) activity was determined using a diagnostic assay kit (Sichuan Maker Science and Technology Co., Ltd., Chengdu, China) by an automated chemistry analyzer (Olympus AU1000, Japan) by Central Laboratory of the Affiliated Jingzhou Hospital of Yangtze University.

Livers were quickly removed, washed with ice-cold PBS, blotted and weighed, and then a tissue homogenate (1% or 10%, w/v) was prepared in normal saline. The homogenates were then centrifuged at 4000 rpm (4 ℃) for 20 min to collect supernatants for determination of SOD and MDA contents at 550 nm and 532 nm, respectively.

Lipid peroxidation was assessed by estimation of MDA in the liver tissues according to the method of Wills (1966). MDA was determined by the thiobarbituric acid assay using a MDA assay kit, according to the manufacturers' instructions. Liver tissue protein was measured using Coomassie Brilliant Blue protein reagent, and MDA content was expressed as nmol/mg protein. SOD activity was determined by measuring the inhibition of formation of NADPH-phenazine methosulphate nitroblue tetrazolium.

Histochemistry

Liver tissues fixed in 10% formalin were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin-eosin.

TUNEL staining

Paraffin-embedded liver tissues were assayed for DNA fragmentation using a terminal deoxynucleotidyl TUNEL reaction, according to the manufacturer's instructions. Slides were developed with diaminobenzidine substrate, counterstained with HE, and then examined for evidence of apoptosis. The number of brown apoptotic cells was normalized to total cells as detected by HE. Four fields of each image were counted.

Reverse transcriptase polymerase chain reaction

RNA was extracted from the livers using TRIzol® Reagent (Invitrogen) according to the manufacturer's instruction. cDNA was synthesized from 2 μg of total RNA using PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara Biotechnology, Co., Ltd., Da Lian, LiaoNing, China). PCR amplifications were performed by standard methods using following specific primers: for Bcl-2: Sense: 5'-GGC ATC TTC TCC TTC CAG-3', Antisense: 5'-CTA CCC AGC CTC CGT TAT-3'; for Bax: Sense: 5'-TTT CAT CCA GGA TCG AGC AGG-3', Antisense: 5'-GCA AAG TAG AAG AGG GCA ACC AC-3'[15].

Statistical analysis

A computer program (SPSS 13.0) was used for statistical analysis. Data were presented as mean ± SE. Student's *t* test (two groups) or one way ANOVA (multiple groups) were used. $P < 0.05$ indicated statistical significance.

RESULTS

CMN treatment attenuates APAP-induced liver injury

To explore the protective effect of CMN on APAP-induced hepatic toxicity, the animals were injected intraperitoneally with CMN (10 or 20 mg/kg body weight) 2 h before APAP (300 mg/kg body weight), and serum ALT was analyzed after 16 h of administration. Compared with PBS control (41.70 ± 2.82 U/L), APAP treatment significantly increased serum ALT levels (5406.80 ± 1785.75 U/L, 126.9-fold of the control, Figure 1A, $P < 0.001$ *vs* control). As expected, CMN pretreatment significantly suppressed the plasma ALT activity in a dose-dependent manner (10 mg/kg: 801.46 ± 661.34 U/L; 20 mg/kg: 99.68 ± 86.48 U/L, Figure 1A, $P < 0.001$ *vs* the model group). This protective effect was further confirmed by analysis of histological findings, as shown in Figure 1B, severe sinusoidal congestion and hemorrhage, inflammatory cell infiltration and gross necrosis were observed in the liver of mice treated with APAP. However, these pathological changes were dramatically suppressed by CMN treatment. To evaluate its potential therapeutic role, CMN was administrated after 2 h of APAP injection, and a marked reduction of serum ALT was also observed (395.40 ± 133.52 U/L, Figure 1A). Of note, CMN alone did not influence serum transaminase (Figure 1A) and urea, creatinine (data not shown) in normal control mice.

CMN pretreatment inhibits production of MDA

As lipid peroxidation has been reported to be closely related to APAP-induced toxicity, the content of malondialdehyde, the end product of lipid peroxidation, in the liver tissues was detected at 16 h after APAP treatment. Only low levels of MDA were observed in the control mice (10.81 ± 1.46 nmol/mg protein), but a significant increase was found in APAP-treated mice (16.03 ± 2.58 nmol/mg protein). As expected, MDA contents were significantly inhibited by 20 mg/kg CMN pretreatment (10.96 ± 0.87 nmol/mg protein, Figure 2).

CMN pretreatment enhances activity of SOD

In addition to lipid peroxidation, oxidative stress is an early events related to radicals generated during the hepatic metabolism of APAP. SOD is an enzyme that neutralizes free radicals[16]. So the activity of SOD was investigated at 16 h after APAP treatment in the liver tissues. A significant decrease in SOD activity was observed in the mice treated with APAP compared with control group (24.54 ± 4.95 U/mg protein *vs* 45.64 ± 5.96 U/mg protein). However, pretreatment of mice with CMN induced a significant increase in the activity of SOD (50.21 ± 1.93 U/mg protein, Figure 3).

CMN pretreatment prevents hepatocyte apoptosis

Based on the above observations, we further explored the possible mechanisms by which CMN attenuates liver injury induced by APAP. Given the importance of apoptosis in APAP-induced liver injury, the extent of hepatocyte apoptosis was determined by TUNEL assay. As shown in Figure 4A, massive hepatocyte apoptosis was detected in the livers of mice treated with APAP. CMN pretreatment markedly prevented the apoptosis induced by APAP. Furthermore, we examined the mRNA expression of anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax in the livers. As shown in Figure 4B, CMN pretreatment down-regulated the mRNA expression of Bax and up-regulated the mRNA expression of Bcl-2 compared with APAP-treated group. These data suggest that CMN inhibits hepatocyte apoptosis *via* regulating the gene expression of Bcl-2 family and then protects the mice from APAP-induced hepatic injury.

DISCUSSION

In the present study, for the first time, we examined the effect of CMN on liver injury induced by APAP and the possible mechanisms in mice. Our data demonstrated that CMN pretreatment dose-dependently alleviated APAP-induced acute liver injury. CMN pretreatment markedly decreased ALT levels in plasma and inhibited the necrosis of hepatocytes in Con A-treated mice. Furthermore, CMN could be also considered as a rescue therapy, for it significantly decreased APAP-induced hepatotoxicity when administered 2 h after APAP overdose.

NAPQI is the reactive metabolite product generated from APAP-induced hepatic toxicity, and it was found to be formed by cytochrome P-450 by a direct two electron oxidation of APAP. At low doses, the metabolite was efficiently detoxified by GSH. However, at high doses, NAPQI leads to GSH depletion and subsequently covalently binds to cysteine residues on proteins, which results in lipid peroxidation reaction[14,17]. As a metabolite of free radical, MDA is generally considered as an impor-

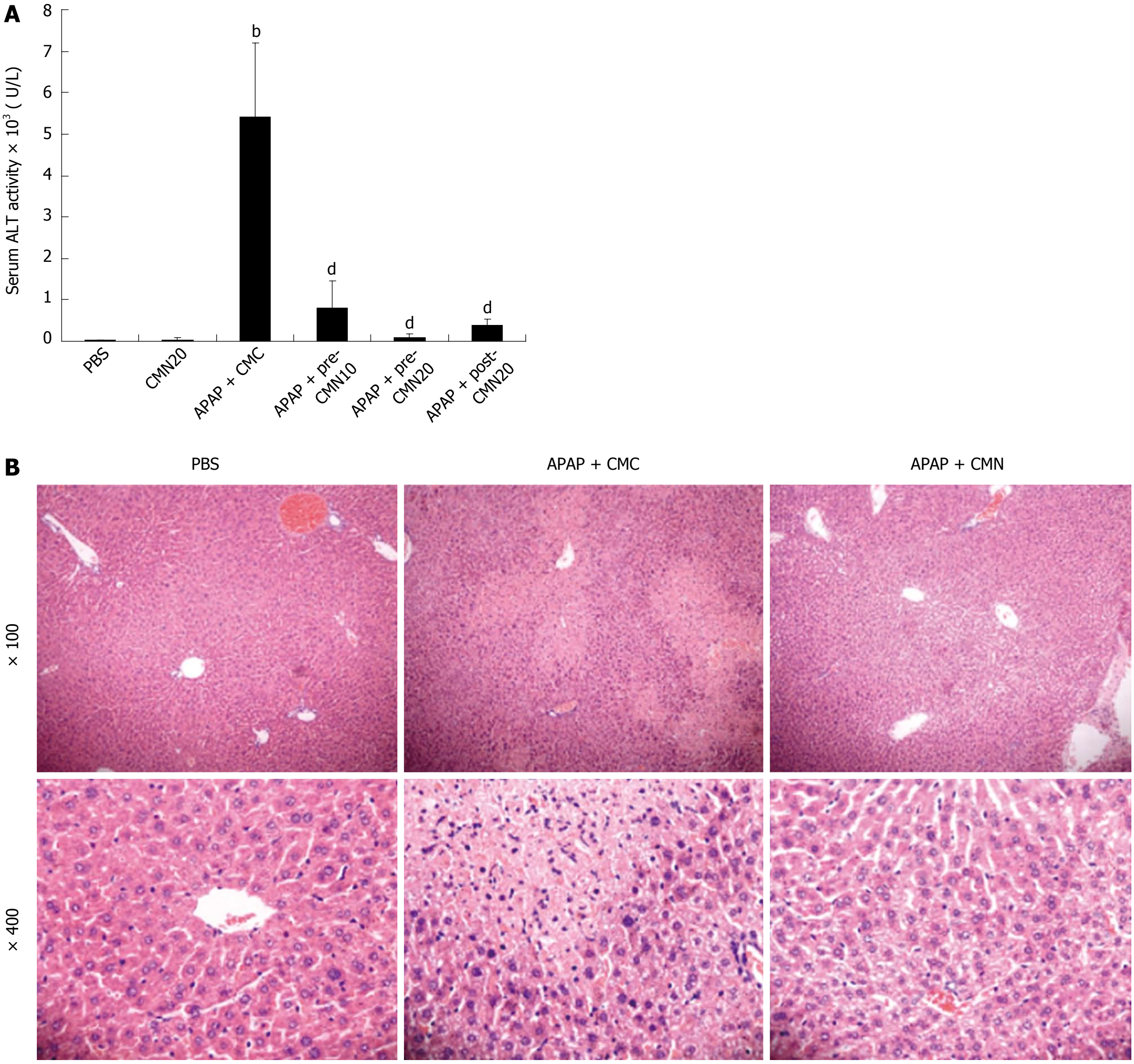

Figure 1 Curcumin treatment protects against acetaminophen-induced hepatic injury in mice. A: Serum alanine aminotransferase (ALT) levels were determined 16 h after acetaminophen injection. Data are expressed as mean ± SE; $n = 10$ mice per group. $^bP < 0.01$ *vs* control; $^dP < 0.001$ *vs* acetaminophen (APAP) + carboxymethylcellulose (CMC); B: Hematoxylin-eosin stained liver sections from animals treated with PBS, APAP + CMC and APAP + CMN (original magnification: × 100 and ×400). Severe inflammatory cell infiltration and gross necrosis of the entire centrilobular areas were obvious in APAP group, and the results were significantly ameliorated in CMN-treated animals. CMN: Curcumin.

tant indicator of lipid peroxidation. In this study, in line with previous reports, we found that MDA in liver tissues was increased significantly 16 h after APAP administration. As expected, our results demonstrated that CMN treatment could inhibit the increase of MDA induced by APAP, suggesting that CMN has potent beneficial effects on lipid peroxidation.

Our organism has a function to neutralize and scavenge the free radical in order to prevent oxidative damage to cells. Such endogenous mechanisms are mainly provided by a set of antioxidant enzymes such as SOD, glutathione peroxidase, and catalase. SOD represents the first line of defense against free radicals, it converts superoxide anion into hydrogen peroxide, and then hydrogen peroxide is converted into oxygen and water by glutathione peroxidase, making reduced GSH as its substrate[18]. As a antioxidant, CMN has been demonstrated to effectively prevent the decrease in SOD activity in a variety of experimental models, including inflammation, cardiotoxicity and carbon tetrachloride-induced liver injury models[19]. In line with these concepts, we found that the major scavenger enzyme SOD activity was significantly decreased in the liver of APAP-treated mice. As expected, pretreatment with CMN restored SOD activity. Therefore, it is suggested that the protective effect of CMN on APAP-induced injury is associated with its inhibition of oxidative stress.

Accumulating evidence suggests that hepatocyte

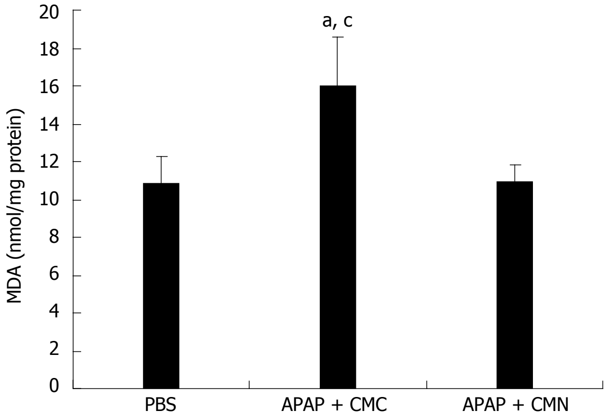

Figure 2 Curcumin pretreatment inhibits malondialdehyde production after acetaminophen induction. Liver homogenate was prepared to analyze the content of malondialdehyde (MDA) 16 h after acetaminophen (APAP) administration. Data are expressed as mean ± SE; n = 10 mice per group. ^aP < 0.05 *vs* control; ^cP < 0.05 *vs* APAP + curcumin (CMN). CMC: Carboxymethylcellulose.

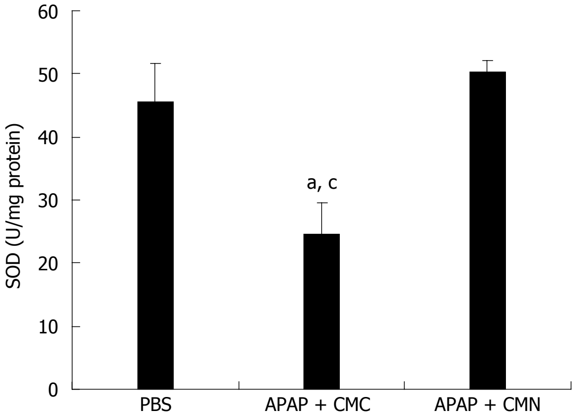

Figure 3 Curcumin pretreatment enhances activity of superoxide dismutase after acetaminophen. Liver homogenate was prepared to analyze the activity of superoxide dismutase (SOD) 16 h after acetaminophen (APAP) administration. Data are expressed as mean ± SE; n = 10 mice per group. ^aP < 0.05 *vs* control; ^cP < 0.05 *vs* APAP + curcumin (CMN). CMC: Carboxymethylcellulose.

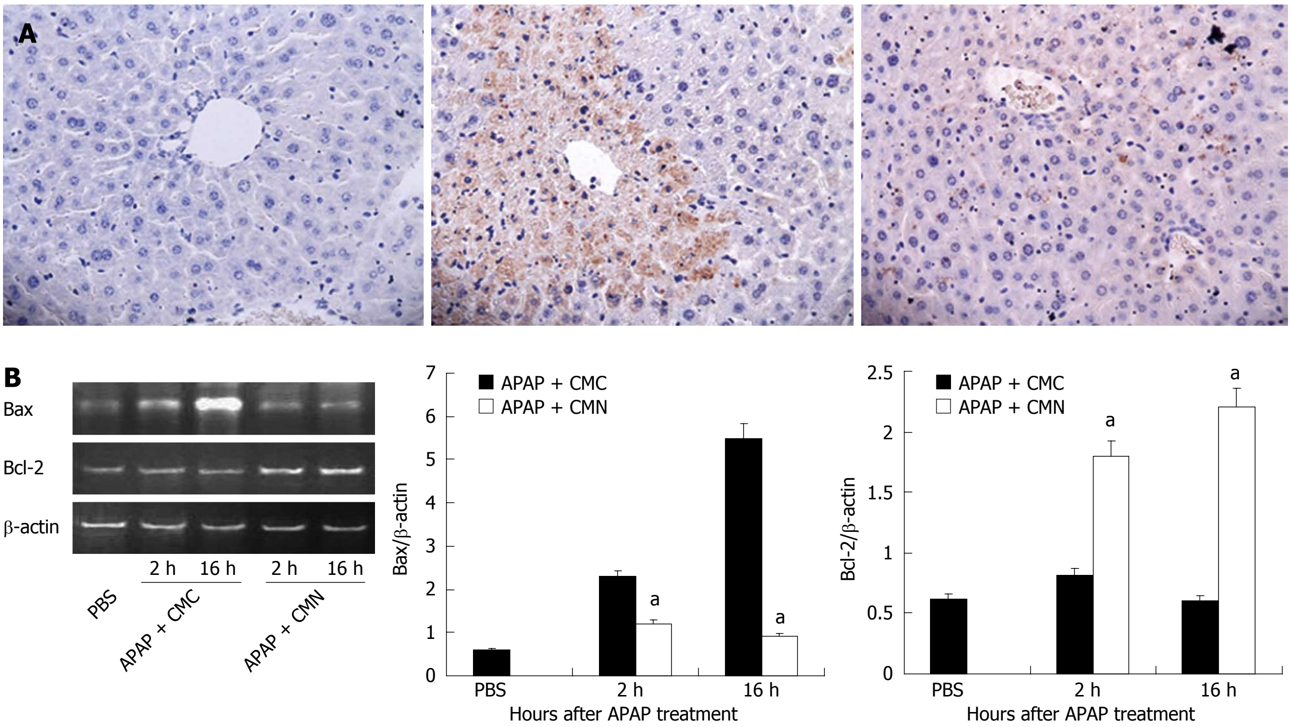

Figure 4 Curcumin pretreatment prevents hepatocyte apoptosis induced by acetaminophen. A: Transferase-mediated dUTP-biotin nick end labeling stained liver sections from animals treated with PBS, acetaminophen (APAP) + carboxymethylcellulose (CMC) and APAP + curcumin (CMN) (original magnification: × 400); B: Liver samples were collected 2 h and 16 h after APAP injection, and the mRNA expression of Bax and Bcl2 was determined by reverse transcriptase polymerase chain reaction. Data are expressed as mean ± SE; n = 6 mice per group. ^aP < 0.05 *vs* APAP + CMC.

apoptosis plays a critical role in APAP-induced hepatic injury, although the mode of cell death inflicted by APAP is still controversial[20,21]. APAP-induced apoptosis is observed not only in primary hepatocytes[22], but also in livers of mice treated with toxic doses of APAP[23]. Also, a recent report showed that hepatic caspase-3 and caspase-9 are activated in both wild type and CXCR2 knock out mice within one hour of APAP treatment[21]. Moreover, inhibiting apoptosis prevents the development of acute liver failure[23]. Based on these concepts, we found that apoptotic hepatocytes were significantly increased in the liver of mice 16 h after APAP treatment and the hepatocyte apoptosis was significantly reduced by CMN pretreatment. Bcl-2 protein is commonly recognized as an anti-apoptotic factor, it inhibits cell apoptosis by preventing mitochondrial membrane depolarization. As a member of the Bcl-2 family, Bax inactivates Bcl-2 by interacting with it to form a heterodimer[24]. In this study, we found that CMN pretreatment down-regulated the mRNA expression of Bax and up-regulated the mRNA expression of Bcl-2 compared with APAP-treated group, suggesting that CMN can increase Bcl-2/Bax ratio, thus reducing APAP-induced apoptosis. The mechanisms of CMN-mediated anti-apoptotic effect remained unclear. A recent study showed that CMN exerts a potent anti-apoptotic effect *via* inhibition of TGF-β as inducer of caspase-3 mediated apoptosis in kidney and lung tissues[25], however, the precise mechanisms by which CMN modulates cell apoptosis in APAP-induced liver injury need to be further investigated.

In summary, our study revealed that CMN has a protective effect on the acute hepatic injury induced by APAP. Both pre- and post-treatment with CMN resulted in a significant reduction in serum ALT and hepatocyte necrosis. The protection of CMN may be related to its inhibition of lipid peroxidation and oxidative stress. Moreover, we found that CMN restored Bcl-2/Bax ratio, thus reducing the APAP-induced hepatocyte apoptosis.

COMMENTS

Background

Acetaminophen (APAP) can cause severe liver damage or even acute liver failure when administered in an acute or cumulative overdose. *N*-acetyl-p-benzoquinone imine (NAPQI) is the metabolite of APAP by cytochrome P450. Accumulation of NAPQI could induce lipid peroxidation, oxidative stress, mitochondrial dysfunction and DNA fragmentation, even liver failure or death. Thus, new and safe preventive measures against APAP-induced hepatic damage are eagerly needed.

Research frontiers

Previous studies have shown that curcumin (CMN) exerts anti-inflammatory, anti-oxidant and anticancer pharmacological activities. APAP lead to liver injury through increasing oxidative stress, lipid peroxidation and pro-apoptosis. In this study, the authors showed that CMN improved hepatic injury through inhibiting oxidative stress, lipid peroxidation and hepatic apoptosis in APAP induced liver damage model.

Innovations and breakthroughs

This study investigated the effect of CMN on the prevention of APAP-induced hepatic toxicity *in vivo* and examined whether CMN affects the production of lipid peroxidation, oxidative stress or hepatocyte apoptosis to attenuate liver damage. The results indicated that CMN could protect mice from APAP-induced liver injury.

Applications

CMN was abundant in the root tubers of *Curcuma longa Linn* and can be purified by modern technology. It has a broad application prospect.

Peer review

It is an interesting study investigating the protection effect of CMN on APAP-caused hepatitis. The experimental evidences presented that APAP induced hepatic injury, with elevated alanine aminotransferase, lipid peroxidation, oxidative stress and apoptosis were improved by CMN.

REFERENCES

1 **Rumack BH**. Acetaminophen misconceptions. *Hepatology* 2004; **40**: 10-15 [PMID: 15239079]

2 **Larson AM**. Acetaminophen hepatotoxicity. *Clin Liver Dis* 2007; **11**: 525-48, vi [PMID: 17723918]

3 **Lee WM**. Acetaminophen and the U.S. Acute Liver Failure Study Group: lowering the risks of hepatic failure. *Hepatology* 2004; **40**: 6-9 [PMID: 15239078]

4 **Chen C**, Hennig GE, Manautou JE. Hepatobiliary excretion of acetaminophen glutathione conjugate and its derivatives in transport-deficient (TR-) hyperbilirubinemic rats. *Drug Metab Dispos* 2003; **31**: 798-804 [PMID: 12756215]

5 **Hinson JA**, Reid AB, McCullough SS, James LP. Acetaminophen-induced hepatotoxicity: role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. *Drug Metab Rev* 2004; **36**: 805-822 [PMID: 15554248]

6 **Zwingmann C**, Bilodeau M. Metabolic insights into the hepatoprotective role of N-acetylcysteine in mouse liver. *Hepatology* 2006; **43**: 454-463 [PMID: 16496303]

7 **Yang R**, Miki K, He X, Killeen ME, Fink MP. Prolonged treatment with N-acetylcystine delays liver recovery from acetaminophen hepatotoxicity. *Crit Care* 2009; **13**: R55 [PMID: 19358737 DOI: 10.1186/cc7782]

8 **Kheradpezhouh E**, Panjehshahin MR, Miri R, Javidnia K, Noorafshan A, Monabati A, Dehpour AR. Curcumin protects rats against acetaminophen-induced hepatorenal damages and shows synergistic activity with N-acetyl cysteine. *Eur J Pharmacol* 2010; **628**: 274-281 [PMID: 19919835 DOI: 10.1016/j.ejphar.2009.11.027]

9 **Joe B**, Vijaykumar M, Lokesh BR. Biological properties of curcumin-cellular and molecular mechanisms of action. *Crit Rev Food Sci Nutr* 2004; **44**: 97-111 [PMID: 15116757]

10 **Rivera-Espinoza Y**, Muriel P. Pharmacological actions of curcumin in liver diseases or damage. *Liver Int* 2009; **29**: 1457-1466 [PMID: 19811613]

11 **Rajakrishnan V**, Jayadeep A, Arun OS, Sudhakaran PR, Menon VP. Changes in the prostaglandin levels in alcohol toxicity: effect of curcumin and N-acetylcysteine. *J Nutr Biochem* 2000; **11**: 509-514 [PMID: 11120449]

12 **Messner DJ**, Sivam G, Kowdley KV. Curcumin reduces the toxic effects of iron loading in rat liver epithelial cells. *Liver Int* 2009; **29**: 63-72 [PMID: 18492020 DOI: 10.1111/j.1478-3231.2008.01793.x]

13 **Reyes-Gordillo K**, Segovia J, Shibayama M, Tsutsumi V, Vergara P, Moreno MG, Muriel P. Curcumin prevents and reverses cirrhosis induced by bile duct obstruction or CCl4 in rats: role of TGF-beta modulation and oxidative stress. *Fundam Clin Pharmacol* 2008; **22**: 417-427 [PMID: 18705752 DOI: 10.1111/j.1472-8206.2008.00611.x]

14 **Yin H**, Cheng L, Holt M, Hail N, Maclaren R, Ju C. Lactoferrin protects against acetaminophen-induced liver injury in mice. *Hepatology* 2010; **51**: 1007-1016 [PMID: 20099297]

15 **Zhang H**, Gong Q, Li JH, Kong XL, Tian L, Duan LH, Tong J, Song FF, Fang M, Zheng F, Xiong P, Tan Z, Gong FL. CpG ODN pretreatment attenuates concanavalin A-induced hepatitis in mice. *Int Immunopharmacol* 2010; **10**: 79-85 [PMID: 19818415 DOI: 10.1016/j.intimp.2009.09.025]

16 **Rahman K**. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging* 2007; **2**: 219-236 [PMID: 18044138]

17 **Hinson JA**, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. *Handb Exp Pharmacol* 2010; (**196**): 369-405 [PMID: 20020268]

18 **Wu YL**, Jiang YZ, Jin XJ, Lian LH, Piao JY, Wan Y, Jin HR, Joon Lee J, Nan JX. Acanthoic acid, a diterpene in Acanthopanax koreanum, protects acetaminophen-induced hepatic toxicity in mice. *Phytomedicine* 2010; **17**: 475-479 [PMID: 19836221 DOI: 10.1016/j.phymed.2009.07.011]

19 **Naik SR**, Thakare VN, Patil SR. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: evidence of its antioxidant property. *Exp Toxicol Pathol* 2011; **63**: 419-431 [PMID: 20363603]

20 **Kass GE**, Macanas-Pirard P, Lee PC, Hinton RH. The role of apoptosis in acetaminophen-induced injury. *Ann N Y Acad Sci* 2003; **1010**: 557-559 [PMID: 15033790]

21 **Hu B**, Colletti LM. CXC receptor-2 knockout genotype increases X-linked inhibitor of apoptosis protein and protects mice from acetaminophen hepatotoxicity. *Hepatology* 2010; **52**: 691-702 [PMID: 20683965 DOI: 10.1002/hep.23715]

22 **Sharma S**, Singh RL, Kakkar P. Modulation of Bax/Bcl-2 and caspases by probiotics during acetaminophen induced

apoptosis in primary hepatocytes. *Food Chem Toxicol* 2011; **49**: 770-779 [PMID: 21130831 DOI: 10.1016/j.fct.2010.11.041]

23 **Hu J**, Yan D, Gao J, Xu C, Yuan Y, Zhu R, Xiang D, Weng S, Han W, Zang G, Yu Y. rhIL-1Ra reduces hepatocellular apoptosis in mice with acetaminophen-induced acute liver failure. *Lab Invest* 2010; **90**: 1737-1746 [PMID: 20644519 DOI: 10.1038/labiNvest.2010.127]

24 **Rajan D**, Wu R, Shah KG, Jacob A, Coppa GF, Wang P. Human ghrelin protects animals from renal ischemia-reperfusion injury through the vagus nerve. *Surgery* 2012; **151**: 37-47 [PMID: 21943641 DOI: 10.1016/j.surg.2011.06.027]

25 **Awad AS**, El-Sharif AA. Curcumin immune-mediated and anti-apoptotic mechanisms protect against renal ischemia/reperfusion and distant organ induced injuries. *Int Immunopharmacol* 2011; **11**: 992-996 [PMID: 21354353 DOI: 10.1016/j.intimp.2011.02.015]

P- Reviewers: Hsu CP, Jung YD **S- Editor**: Zhai HH
L- Editor: Ma JY **E- Editor**: Zhang DN

WJG

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7447

World J Gastroenterol 2013 November 14; 19(42): 7447-7454
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Prognostic factors in non-malignant and non-cirrhotic patients with portal cavernoma: An 8-year retrospective single-center study

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Received: June 6, 2013 **Revised:** August 1, 2013
Accepted: August 28, 2013
Published online: November 14, 2013

Abstract

AIM: To evaluate the outcome of non-malignant and non-cirrhotic patients with portal cavernoma and to determine the predictors for survival.

METHODS: Between July 2002 and June 2010, we retrospectively enrolled all consecutive patients admitted to our department with a diagnosis of portal cavernoma without abdominal malignancy or liver cirrhosis. The primary endpoint of this observational study was death and cause of death. Independent predictors of survival were identified using the Cox regression model.

RESULTS: A total of 64 patients were enrolled in the study. During a mean follow-up period of 18 ± 2.41 mo, 7 patients died. Causes of death were pulmonary embolism (n = 1), acute leukemia (n = 1), massive esophageal variceal hemorrhage (n = 1), progressive liver failure (n = 2), severe systemic infection secondary to multiple liver abscesses (n = 1) and accident (n = 1). The cumulative 6-, 12- and 36-mo survival rates were 94.9%, 86% and 86%, respectively. Multivariate Cox regression analysis demonstrated that the presence of ascites (HR = 10.729, 95%CI: 1.209-95.183, P = 0.033) and elevated white blood cell count (HR = 1.072, 95%CI: 1.014-1.133, P = 0.015) were independent prognostic factors of non-malignant and non-cirrhotic patients with portal cavernoma. The cumulative 6-, 12- and 36-mo survival rates were significantly different between patients with and without ascites (90%, 61.5% and 61.5% *vs* 97.3%, 97.3% and 97.3%, respectively, P = 0.0008).

CONCLUSION: The presence of ascites and elevated white blood cell count were significantly associated with poor prognosis in non-malignant and non-cirrhotic patients with portal cavernoma.

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Key words: Extrahepatic portal vein obstruction; Portal cavernoma; Survival; Prognostic factors; Ascites

Core tip: Little is known regarding the prognostic factors of non-malignant and non-cirrhotic patients with portal cavernoma. We conducted a retrospective single-center study of 64 patients admitted to our department between July 2002 and June 2010 to evaluate this issue. Multivariate Cox regression analysis demonstrated that the presence of ascites was an independent prognostic factor of non-malignant and non-cirrhotic patients with portal cavernoma.

Qi XS, Bai M, He CY, Yin ZX, Guo WG, Niu J, Wu FF, Han GH.

Prognostic factors in non-malignant and non-cirrhotic patients with portal cavernoma: An 8-year retrospective single-center study. *World J Gastroenterol* 2013; 19(42): 7447-7454 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7447.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7447

INTRODUCTION

Portal cavernoma, also known as cavernous transformation of the portal vein, is a rare entity, which is characterized by a tangle of tortuous hepatopetal collateral veins in the hilum[1]. It is traditionally considered a sequela of extrahepatic portal vein obstruction (EHPVO) to compensate for the interrupted portal blood flow[2,3]. Current treatment strategies for portal cavernoma focus on the prevention and treatment of variceal hemorrhage, prevention of recurrent thrombosis, and treatment of symptomatic portal biliopathy[4-6]. Given the rarity of portal cavernoma, controlled studies are unavailable, and therapeutic options vary in different centers. Previous studies, in which malignancy and cirrhosis were not excluded, have revealed that the increased mortality in EHPVO patients is closely associated with advanced age, presence of malignancy and cirrhosis, high bilirubin and deterioration of liver function[7-9]. However, little information is known regarding the prognostic factors in non-malignant and non-cirrhotic patients with portal cavernoma due to its low morbidity and mortality.

We conducted a retrospective study to determine the predictors for survival of non-malignant and non-cirrhotic patients with portal cavernoma managed by a uniform therapeutic strategy at our center.

MATERIALS AND METHODS

Study design

Between July 2002 and June 2010, all consecutive patients with a diagnosis of portal cavernoma without abdominal malignancy or liver cirrhosis who were admitted to our department were enrolled in this observational study[10-12], regardless of age. Baseline data were collected upon admission or referral. Regular blood tests, hepatic and renal function tests, prothrombin time, internationalized normalized ratio (INR), color Doppler ultrasound (CDUS), computed tomography (CT) and endoscopy were performed in all patients. Thrombotic risk factors of EHPVO, including *JAK2 V617F* mutation, CD55 and CD59 deficiencies, anti-cardiolipin IgG antibodies, and factor V Leiden or prothrombin gene G20210A mutation, were detected at our department after September 2009[13,14]. Additionally, we recorded the date of diagnosis of portal cavernoma at our own hospital or an outside facility. Follow-up data were obtained every six months through outpatient or phone conversations with the patient or his or her family members. The primary endpoint was death. Follow-up continued either until death or July 2010. The study protocol was approved by the ethics committee of our hospital.

Diagnosis and definitions

Portal cavernoma was characterized by a tangle of tortuous hepatopetal collateral veins in the hilum. An acute thrombotic episode was defined as fulfillment of both of the following criteria: (1) recent onset of abdominal pain; and (2) a high intraluminal density within the portal vein on non-enhanced CT scans, while contrast-enhanced CT scans displayed cavernous vessels around the obstructed portal vein[15,16].

As previously described, the degree of portal venous obstruction was classified as partial obstruction, complete obstruction and fibrotic cord instead of original main portal vein[12,17,18]. The extent of obstruction within the portal venous system was also evaluated.

Liver cirrhosis and hepatocellular carcinoma were excluded on the basis of a history of chronic liver disease, clinical presentation, liver function, alpha-fetoprotein and positive findings on imaging (*i.e.*, ultrasound and CT scans)[18]. A liver biopsy was obtained, if a diagnosis of cirrhosis was inconclusive or if hepatocellular carcinoma was suspected. Other abdominal malignancy was excluded by imaging.

The degree of variceal size was based on the general rules established by the Japanese Research Society for Portal Hypertension (low-risk varices: F1 or F2 with negative red color sign; high-risk: F1 or F2 with positive red color sign, or F3 irrespective of red color sign)[19]. The presence of ascites was diagnosed by physical examination, ultrasound and CT scans. The grade of ascites was based on the definitions of the International Ascites Club (grade Ⅰ: mild ascites only detectable by ultrasound; grade Ⅱ: moderate symmetrical abdominal distension; grade Ⅲ: marked abdominal distension)[20].

Therapeutic strategy

Our therapeutic strategy was aimed at minimal invasiveness and maximal beneficial effects through symptom resolution.

After diagnosis of an acute thrombotic episode, the patients received a continuous intravenous infusion of unfractionated heparin followed by oral warfarin. Initially, heparin was regularly administered intravenously at a starting dose of 1000-1400 U/h for 5 d. Subsequently, oral warfarin was prescribed at the dosage of 2.5-5 mg/d for at least 6 mo and was adjusted to maintain the INR at a target of 2.5 (range 2.0-3.0)[21]. A three-day overlap between intravenous and oral anticoagulation was required. Life-long oral anticoagulants were prescribed to patients with thrombophilia. If abdominal pain was progressive, we either indirectly infused thrombolytic agents into the superior mesenteric artery or performed direct thrombolysis in the portal vein by a percutaneous transhepatic approach. If ischemic intestinal infarction was diagnosed, emergency bowel resection was performed.

Once acute variceal bleeding was diagnosed, medical or endoscopic therapy was adopted as the first-line treatment option. If active bleeding was uncontrolled or if repeated hospitalizations were necessary to control recurrent variceal bleeding, a transjugular intrahepatic portosystemic shunt (TIPS) insertion was performed through a transjugular approach alone or in combination with a transhepatic or transsplenic approach, as previously described[12,18,22]. If a TIPS procedure failed or was refused, splenectomy and devascularization were considered.

Other symptomatic treatments included anticoagulation for prevention of recurrent thrombosis, diuretics and/or paracentesis plus albumin for grade Ⅱ and Ⅲ ascites, splenectomy for hypersplenism and massive splenomegaly and prophylactic endoscopic therapy for high-risk varices. Additionally, if patients presented with a long history of repeated gastrointestinal syndromes unresponsive to conservative therapies, the TIPS procedure was considered.

Statistical analysis

Quantitative data were reported as mean ± SE and were compared with the independent sample t test or one-way analysis of variance; qualitative data were reported as frequencies and were compared with the χ^2 test or the Fisher exact test, as appropriate. Cumulative survival rates were assessed by the Kaplan-Meier curves and were compared with a log-rank test. Independent predictors of survival were identified using the Cox regression model. The covariates incorporated into the multivariate analysis were the variables that reached statistical significance ($P < 0.05$) in univariate analysis. Two-tailed P values <0.05 were considered statistically significant. All statistical calculations were performed with SPSS 12.0 (Chicago, IL, United States).

RESULTS

Characteristics of patients

A total of 64 patients diagnosed with portal cavernoma without liver cirrhosis or abdominal malignancy were enrolled in the study. Notably, 5 patients presented with cavernous collateral veins around a patent main portal vein[10]. Baseline patient characteristics are summarized in Table 1.

Table 1 Patient characteristics at admission

Sex (female/male)	26/38
Age at admission (yr)	32.05 ± 2.00
Age at first diagnosis of portal cavernoma (≤ 18 yr/> 18 yr)	26/38
History of portal cavernoma (yr)	3.68 ± 0.78
Clinical presentations	
Abdominal distension	37
Abdominal pain	26
Variceal bleeding	29
Degree of varices (high risk/low risk/no varices)	6/12/1946
Ascites	20
Degree of ascites (grade Ⅲ/grade Ⅱ/grade Ⅰ/no ascites)	3/1/16/44
Hydrothorax	10
Laboratory tests	
RBC (10^{12}/L)	3.95 ± 0.11
Hb (g/L)	106.25 ± 4.00
WBC (10^9/L)	8.11 ± 1.03
PLT (10^9/L)	298.73 ± 33.67
PT (s)	14.14 ± 0.24
INR	1.15 ± 0.02
ALT (U/L)	29.97 ± 3.31
AST (U/L)	30.28 ± 2.64
ALP (U/L)	107.21 ± 7.68
GGT (U/L)	41.16 ± 5.96
ALB (g/L)	38.25 ± 0.58
TBIL (μmol/L)	16.05 ± 1.32
Serum Cr (μmol/L)	70.65 ± 2.48
Serum Na (mmol/L)	139.54 ± 0.45
Child-Pugh class (A/B/C)	12/1/1951
Child-Pugh score	5.70 ± 0.13
MELD score	4.25 ± 0.53
Location and degree of obstruction	
Main portal vein (patent/partial/complete/fibrotic cord)	5/5/12/42
Right portal vein obstruction	49
Left portal vein obstruction	51
Splenic vein obstruction and splenectomy	39
Superior mesenteric vein obstruction	34

Data are expressed as quantitative data as frequency or mean ± SE. RBC: Red blood cell; Hb: Hemoglobin; WBC: White blood cell; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl transferase; ALB: Albumin; TBIL: Total bilirubin.

Possible risk factors

Thrombotic risk factors of EHPVO were detected in 33 patients. Among them, 11 had positive *JAK2 V617F* mutation, none had both CD55 and CD59 deficiencies, two had weakly positive anti-cardiolipin IgG antibodies, and none had positive factor Ⅴ Leiden or prothrombin gene *G20210A* mutation. Previous history of infection before admission included colitis ($n = 1$), pelvic infection ($n = 1$), appendicitis ($n = 1$), intra-abdominal infection secondary to duodenal ulcer perforation ($n = 1$), umbilical cord infection ($n = 2$), megacolon disease of newborn ($n = 1$), bacterial dysentery ($n = 1$), and pancreatitis ($n = 4$). Previous history of abdominal surgery before admission included splenectomy and devascularization for variceal bleeding ($n = 11$), splenectomy for hypersplenism and/or splenomegaly ($n = 8$), splenectomy for traumatic spleen rupture ($n = 1$), partial splenic artery embolization for hypersplenism ($n = 1$), cholecystectomy ($n = 4$), surgical repair of peptic ulcer perforation ($n = 1$), total hysterectomy for hysteromyoma ($n = 1$), and cesarean delivery ($n = 1$). Notably, 7 and 13 patients underwent splenectomy before and after the diagnosis of portal cavernoma, respectively.

Clinical profile of patients with variceal bleeding

Compared to patients without variceal bleeding, those with variceal bleeding were younger at admission (37.54 ± 2.56 years in the non-bleeding group *vs* 25.41 ± 2.74 years in the bleeding group, $P = 0.002$) and had a longer history of portal cavernoma (1.86 ± 0.66 years in the non-bleeding group *vs* 5.89 ± 1.44 years in the bleeding group, $P = 0.009$). A fibrotic cord replacing the main portal vein was more frequently found on CT scans in pa-

Table 2 Clinical profile in patients with and without variceal bleeding

Variables	Variceal bleeding (n = 29)	No variceal bleeding (n = 35)	P value
Age at admission (yr)	25.41 ± 2.74	37.54 ± 2.56	0.002
Sex (female/male)	14/15	12/23	0.257
History of portal cavernoma (yr)	5.89 ± 1.44	1.86 ± 0.66	0.009
Abdominal distension (yes/no)	11/18	26/9	0.003
Abdominal pain (yes/no)	3/26	23/12	< 0.001
Ascites (yes/no)	8/21	12/23	0.565
Hydrothorax (yes/no)	4/25	6/29	0.713
Jaundice (yes/no)	2/27	2/33	0.846
RBC (10^{12}/L)	3.36 ± 0.12	4.44 ± 0.12	< 0.001
Hb (g/L)	87.21 ± 4.60	122.03 ± 4.86	< 0.001
WBC (10^9/L)	7.47 ± 1.82	8.65 ± 1.16	0.574
PLT (10^9/L)	317.07 ± 50.27	283.53 ± 45.84	0.624
PT (s)	14.08 ± 0.35	14.18 ± 0.34	0.838
INR	1.15 ± 0.03	1.15 ± 0.03	0.946
ALT (U/L)	26.38 ± 4.87	32.94 ± 4.51	0.327
AST (U/L)	28.85 ± 4.28	30.93 ± 3.33	0.388
AST/ALT	1.23 ± 0.08	1.21 ± 0.11	0.874
ALP (U/L)	115.98 ± 13.43	99.94 ± 8.57	0.302
GGT (U/L)	31.69 ± 8.60	49.00 ± 8.12	0.150
ALB (g/L)	37.23 ± 0.79	39.09 ± 0.82	0.111
TBIL (μmol/L)	15.54 ± 2.13	16.48 ± 1.66	0.724
DBIL (μmol/L)	7.07 ± 1.54	7.43 ± 0.98	0.838
Serum Cr (μmol/L)	70.17 ± 3.88	71.06 ± 3.26	0.860
Serum Na (mmol/L)	139.26 ± 0.55	139.77 ± 0.70	0.577
Child-Pugh class (A/B/C)	23/5/1	28/7/0	0.529
Child-Pugh score	5.69 ± 0.21	5.71 ± 0.18	0.928
MELD score	3.93 ± 0.88	4.51 ± 0.65	0.593
Main portal vein (fibrotic cord/complete obstruction/partial obstruction/patency)	27/0/1/1	15/12/3/4	< 0.001
Right portal vein (obstruction/patency)	19/10	30/5	0.058
Left portal vein (obstruction/patency)	21/8	30/5	0.188
Splenic vein (obstruction and splenectomy/patency)	14/15	25/10	0.059
Superior mesenteric vein (obstruction/patency)	11/18	23/12	0.027

Data are expressed as quantitative data as frequency or mean ± SE. RBC: Red blood cell; Hb: Hemoglobin; WBC: White blood cell; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl transferase; ALB: Albumin; TBIL: Total bilirubin; DBIL: Direct bilirubin; MELD: Model for end-stage liver disease.

tients with variceal bleeding than those without (27/29 *vs* 15/35, P < 0.001). In contrast, superior mesenteric vein obstruction was less frequently observed in patients with variceal bleeding than those without (11/29 *vs* 23/35, P = 0.027). No significant relationship between Child-Pugh score and variceal bleeding was observed (Table 2).

Clinical profile of patients with ascites

The white blood cell (WBC) count, prothrombin time and INR were significantly higher, and albumin and serum sodium were significantly lower in patients with ascites than in those without (Table 3). A higher Child-Pugh score was also present in patients with ascites than in those without (6.85 ± 0.26 *vs* 5.18 ± 0.07, P < 0.001). Additionally, superior mesenteric vein obstruction was more frequently observed in patients with ascites than in those without (15/20 *vs* 19/44, P = 0.027).

Table 3 Clinical profile in the patients with and without ascites

Variables	Ascites (n = 20)	No ascites (n = 44)	P value
Age at admission (yr)	36.60 ± 3.99	29.98 ± 2.25	0.127
Sex (female/male)	7/13	19/25	0.537
History of portal cavernoma (yr)	2.38 ± 0.89	4.28 ± 1.06	0.264
Variceal bleeding (yes/no)	12/8	21/23	0.565
Varices (high risk/low risk/no varices)	32/3/9	14/3/3	0.545
Abdominal distension (yes/no)	17/3	20/24	0.003
Abdominal pain (yes/no)	9/11	17/27	0.631
Hydrothorax (yes/no)	10/10	0/44	< 0.001
Jaundice (yes/no)	2/18	2/42	0.403
RBC (10^{12}/L)	3.94 ± 0.21	3.96 ± 0.13	0.944
Hb (g/L)	104.60 ± 6.55	107.00 ± 5.04	0.783
WBC (10^9/L)	12.28 ± 2.83	6.22 ± 0.62	0.006
PLT (10^9/L)	275.45 ± 53.28	309.30 ± 42.86	0.645
PT (s)	15.08 ± 0.41	13.71 ± 0.28	0.007
INR	1.23 ± 0.03	1.11 ± 0.02	0.006
ALT (U/L)	24.65 ± 3.36	32.39 ± 4.54	0.282
AST (U/L)	28.85 ± 4.28	30.93 ± 3.33	0.717
AST/ALT	1.33 ± 0.18	1.17 ± 0.07	0.305
ALP (U/L)	103.55 ± 10.29	108.88 ± 10.21	0.751
GGT (U/L)	46.70 ± 10.00	38.64 ± 7.43	0.535
ALB (g/L)	35.17 ± 0.91	39.65 ± 0.64	< 0.001
TBIL (μmol/L)	17.79 ± 2.92	15.27 ± 1.39	0.379
DBIL (μmol/L)	8.35 ± 1.57	6.78 ± 1.05	0.409
Serum Cr (μmol/L)	73.40 ± 5.16	69.40 ± 2.77	0.460
Serum Na (mmol/L)	138.01 ± 1.00	140.24 ± 0.45	0.021
Child-Pugh class (A/B/C)	8/11/1	43/1/0	< 0.001
Child-Pugh score	6.85 ± 0.26	5.18 ± 0.07	< 0.001
MELD score	5.60 ± 0.93	3.64 ± 0.63	0.086
Main portal vein (obstruction/patency)	18/2	40/3	0.908
Right portal vein (obstruction/patency)	15/5	34/10	0.842
Left portal vein (obstruction/patency)	16/4	35/9	0.967
Splenic vein (obstruction and splenectomy/patency)	11/9	28/16	0.512
Superior mesenteric vein (obstruction/patency)	15/5	19/25	0.018

Data are expressed as quantitative data as frequency or mean ± SE. RBC: Red blood cell; Hb: Hemoglobin; WBC: White blood cell; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl transferase; ALB: Albumin; TBIL: Total bilirubin; DBIL: Direct bilirubin; MELD: Model for end-stage liver disease.

Treatment

Ten patients were diagnosed with acute thrombotic episodes. None presented with variceal bleeding, but four had high-risk varices detected by endoscopy. Mild ascites was found by CDUS in three patients. The main portal vein was completely (n = 9) or partially (n = 1) obstructed. After intravenous anticoagulation was administered for 2-5 d, abdominal pain was alleviated in 5 patients and aggravated in another 5 patients. For the 5 patients with improved abdominal pain, oral anticoagulation was continued. During follow-up, partial recanalization of the main portal vein was found in 1 patient, while the main portal vein became unidentifiable and was replaced by cavernous collateral vessels in the remaining 4 patients. One patient with high-risk varices experienced melena 2 wk after anticoagulation. Anticoagulants were discontinued in this patient and were not resumed. For the 5 patients with increased abdominal pain, thrombolytics were indirectly infused *via* the superior mesenteric artery in 3 patients and directly *via* the portal vein in two patients. Of the 3 patients who received indirect thrombolysis, one became asymptomatic, while the other 2 patients underwent intestinal resection for ischemic intestinal infarction. One of the 2 patients died of pulmonary embolism 5 d after surgery. Of the 2 patients receiving direct thrombolysis for 3-5 d, abdominal pain completely resolved. During follow-up, partial recanalization of the main portal vein was found in 2 patients, while the main portal vein was replaced by cavernous collateral vessels in the remaining 3 patients. No adverse events were recorded.

Twenty-nine patients presented with acute (n = 7) and recurrent variceal bleeding (n = 22). Of the patients with acute variceal bleeding, 5 received pharmacological treatment, 1 had emergency endoscopic sclerotherapy and one underwent embolization of the gastric varices *via* a percutaneous trans-splenic approach. Active bleeding was controlled in these patients. One patient died of massive variceal rebleeding 49 d after discharge. Of the patients with recurrent variceal bleeding, TIPS placement was attempted after admission and was technically successful in eight patients. Of the remaining 14 patients with TIPS failure, 13 experienced at least one episode of rebleeding within 6 mo, and 1 was lost to follow-up. Three of these 13 patients underwent splenectomy and devascularization, and 10 had repeated endoscopic treatments.

Additionally, TIPS procedures were attempted in 4 patients who presented with repeated episodes of abdominal distension over more than 4 years, and these were technically successful in three patients. After successful TIPS insertions, two patients with SMV thrombosis developed shunt occlusions, and one of them presented with variceal hemorrhage that was controlled by endoscopic sclerotherapy. The shunt was patent in another patient. Preoperatively, his spleen was palpably enlarged 10 cm below the left costal margin. The size of the spleen was reduced, but the spleen remained clinically palpable at 20 mo after surgery. After TIPS failure, one patient underwent splenectomy for splenomegaly (6 cm below the left costal margin) and hypersplenism (a low WBC count of 1520 cells/mm^3 and a low platelet count of 32000/mm^3).

Overall survival

After a mean follow-up of 18 ± 2.41 mo, a total of seven patients had died. Causes of death were pulmonary embolism (n = 1), acute leukemia (n = 1), massive esophageal variceal hemorrhage (n = 1), progressive liver failure (n = 2), multiple liver abscesses (n = 1) and accident (n = 1). Overall, 6-, 12- and 36-mo cumulative survival rates were 94.9%, 86% and 86%, respectively (Figure 1A). Cumulative 6-, 12- and 36-mo survival rates were similar between patients with and without variceal bleeding (92.8%, 80.2% and 80.2% *vs* 97.1%, 92.5% and 92.5%, respectively, P = 0.269, Log-rank test) (Figure 1B). Cumulative 6-, 12- and 36-mo survival rates were significantly different between patients with and without ascites (90%, 61.5% and 61.5% *vs* 97.3%, 97.3% and 97.3%, respectively, P = 0.0008, Log-rank test, Figure 1C).

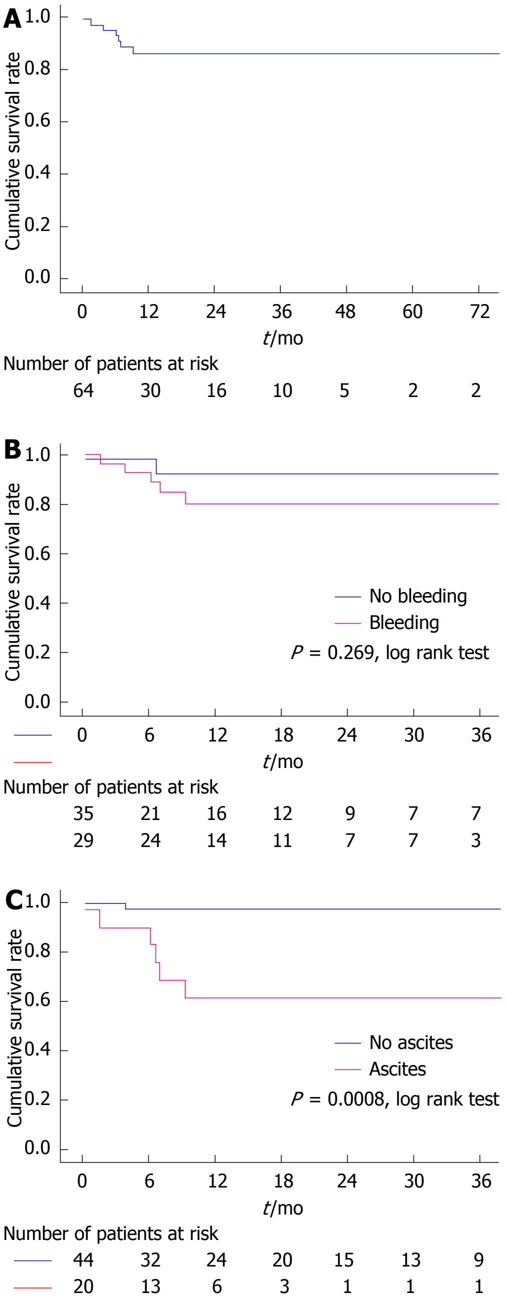

Figure 1 Overall survival in all patients (A), patients with and without variceal bleeding (B) and patients with and without ascites (C).

Table 4 Univariate analysis of baseline variables predicting overall survival

Variables	HR	95%CI	*P* value
Sex (female/male)	0.559	0.108-2.883	0.487
Age at admission	1.032	0.984-1.083	0.194
Age at first diagnosis (≤ 18 yr/> 18 yr)	0.840	0.187-3.772	0.820
History of portal cavernoma	0.989	0.878-1.114	0.857
Varices (yes/no)	0.428	0.083-2.213	0.311
Variceal bleeding (yes/no)	2.450	0.475-12.653	0.285
Abdominal distension (yes/no)	2.253	0.435-11.657	0.333
Abdominal pain (yes/no)	2.010	0.450-8.989	0.361
Ascites (yes/no)	15.066	1.811-125.337	0.012
Hydrothorax (yes/no)	6.638	1.452-30.346	0.015
RBC	0.681	0.288-1.614	0.383
Hb	0.987	0.964-1.011	0.302
PLT	1.000	0.997-1.003	0.900
WBC	1.099	1.040-1.162	0.001
PT	1.083	0.751-1.562	0.668
INR	5.253	0.057-480.003	0.471
ALT	0.970	0.915-1.029	0.312
AST	0.966	0.905-1.032	0.305
AST/ALT	2.328	0.891-6.083	0.085
ALP	1.003	0.993-1.013	0.558
GGT	1.003	0.989-1.017	0.684
ALB	0.932	0.795-1.094	0.392
TBIL	1.028	0.969-1.091	0.358
DBIL	1.036	0.941-1.140	0.470
Serum Cr	1.007	0.971-1.045	0.704
Serum Na	0.764	0.635-0.918	0.004
Child-Pugh score	1.573	0.976-2.535	0.063
Child-Pugh class (class A/class B and C)	0.083	0.009-0.763	0.028
MELD score	1.067	0.899-1.267	0.459
TIPS insertion (failure/success)	0.677	0.095-4.806	0.696
Main portal vein (patency/obstruction)	0.042	0.0001-1720.16	0.558
Main portal vein (no fibrotic cord/fibrotic cord)	0.798	0.155-4.113	0.787
Right portal vein (patency/obstruction)	2.269	0.507-10.151	0.284
Left portal vein (patency/obstruction)	1.639	0.318-8.455	0.555
Splenic vein (patency/obstruction and splenectomy)	0.533	0.103-2.766	0.454
Superior mesenteric vein (patency/obstruction)	0.159	0.019-1.330	0.090

RBC: Red blood cell; Hb: Hemoglobin; WBC: White blood cell; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl transferase; ALB: Albumin; TBIL: Total bilirubin; DBIL: Direct bilirubin; MELD: Model for end-stage liver disease; TIPS: Transjugular intrahepatic portosystemic shunt.

Given that the risk factors of EHPVO were detected in only half of the patients, they were not included in the prognostic analysis. In the univariate analysis, variables that were significantly associated with reduced survival included the presence of ascites and hydrothorax, a low level of serum sodium, an elevated WBC count and a high Child-Pugh class (Table 4). Multivariate backward stepwise Cox regression analysis demonstrated that the presence of ascites (HR = 10.729, 95%CI: 1.209-95.183, P = 0.033) and an elevated WBC count (HR = 1.072, 95%CI: 1.014-1.133, P = 0.015) were independent predictors of increased mortality in non-malignant and non-cirrhotic patients with portal cavernoma.

DISCUSSION

This study was primarily designed to evaluate the outcome of non-malignant and non-cirrhotic patients with portal cavernoma and to determine prognostic factors. Our study demonstrated that most patients with portal cavernoma in the absence of cirrhosis or malignancy had a relatively benign course (overall cumulative survival rate was 86% at 60 mo), which is similar to the excellent outcomes of non-cirrhotic patients with portal vein or splanchnic vein thrombosis reported by previous studies[23-25]. However, it should be noted that all deaths occurred within the first year after admission, which is explained by the following points. First, a long history of portal cavernoma was recorded in some patients. It is possible that the underlying disease or liver dysfunction had already become severe in these patients, warranting referral to our highly specialized center. Second, as shown previously, the overall mortality of splanchnic vein thrombosis patients with intestinal infarction is high[23]. As a primary cause of death, intestinal infarction and its secondary severe complications often occur at the early stage. In our study, one of two patients who underwent bowel resection for intestinal infarction died 5 d after surgery.

Despite the high prevalence of variceal bleeding in our patients, the incidence of death was not significant (only one patient died of massive variceal bleeding), mainly due to advances in treatment modalities for controlling bleeding and well-preserved liver function in these patients. More importantly, we found that the presence of ascites might act as the most important prognostic factor for death. This finding could be explained by the higher incidence of liver dysfunction in patients with ascites. Indeed, the deterioration of liver function is caused not only by the presence of ascites itself, but also by a higher prothrombin time and INR and a lower level of serum albumin and sodium, which are closely correlated with the presence of ascites. Based on the prognostic significance of ascites in non-malignant and non-cirrhotic patients with portal cavernoma, therapeutic decision making needs to be further altered. Once ascites is detected, we should pay more attention to early diagnosis and treatment of underlying comorbidities and liver dysfunction. Accordingly, we hypothesize that the prevention and treatment of liver dysfunction should be incorporated into the treatment strategy for portal cavernoma[4].

We also found that an elevated WBC count was an independent predictor of survival. This might be explained by the fact that comorbidities, such as acute leukemia (n = 1) and multiple liver abscesses (n = 1) could be more common in patients with an elevated WBC count. However, it should be noted that the effect size was very small (hazard ratio was very close to 1). Therefore, the significance of WBC count on patient survival might be clinically slight.

Our study has several limitations. First, only patients diagnosed with portal cavernoma were included in this study. The inclusion criteria may influence the application of prognostic factors in patients with acute EHPVO. However, we believe that it was important to differentiate between the outcomes of acute EHPVO and portal cavernoma, because of their dissimilar clinical presentations and natural history. Second, the prevalence and significance of underlying etiological factors in patients with EHPVO are discussed elsewhere[13,14,26,27], but not in this study. Therefore, we can not demonstrate the association between survival and prothrombotic factors, including acquired and inherited factors. Further work is warranted to explore the effect of etiological factors on survival. Third, given the excellent outcome of non-malignant and non-cirrhotic patients with portal cavernoma, mean follow-up time was relatively short and a low proportion of patients met the endpoints in our study. This bias might miss other potential prognostic factors. An extended follow-up should be carried out in future studies. Fourth, the laboratory analysis of ascitic fluids was not performed. We could not exclude the possibility of spontaneous bacterial peritonitis, especially in cases with an elevated WBC. Finally, given that the number of deaths was only 7, the multivariate Cox regression analysis might be inappropriate. Indeed, the number of variables included in the multivariate analysis might introduce the risk of overfitting the data, thereby leading to a high risk of false positive results. Therefore, the conclusions of this analysis should be taken with caution and further confirmed in larger studies.

In conclusion, our study suggests that the presence of ascites and an elevated WBC count are significantly associated with increased mortality in non-malignant and non-cirrhotic patients with portal cavernoma. Further studies are needed to confirm the prognostic significance of ascites in these patients and to establish a new therapeutic strategy based on the presence of ascites.

COMMENTS

Background

Portal cavernoma is traditionally considered a sequela of extrahepatic portal vein obstruction (EHPVO) to compensate for the interrupted portal blood flow. Due to its low morbidity and mortality in non-malignant and non-cirrhotic patients, little information is known regarding the prognostic factors of portal cavernoma.

Research frontiers

Previous studies, in which malignancy and cirrhosis were not excluded, have revealed that the increased mortality in EHPVO patients is closely associated with advanced age, presence of malignancy and cirrhosis, high bilirubin and deterioration of liver function.

Innovations and breakthroughs

The authors conducted an 8-year retrospective single-center study to determine the predictors for survival of non-malignant and non-cirrhotic patients with portal cavernoma managed by a uniform therapeutic strategy at our center.

Applications

The authors found that the presence of ascites might act as the most important prognostic factor for death in non-malignant and non-cirrhotic patients with portal cavernoma. Based on the prognostic significance of ascites in non-malignant and non-cirrhotic patients with portal cavernoma, therapeutic decision making needs to be further altered.

Terminology

Portal cavernoma, also known as cavernous transformation of the portal vein, is a rare entity, which is characterized by a tangle of tortuous hepatopetal collateral veins in the hilum.

Peer review

It is an interesting review of portal cavernoma evolution. In this study, the authors investigated the prognostic factors for portal cavernoma in non-malignant and non-cirrhotic patients. They were able to show that the presence of ascites and an elevated white blood cell count were strongly associated with poor prognosis in non-cirrhotic patients with portal cavernoma.

REFERENCES

1 **Ohnishi K**, Okuda K, Ohtsuki T, Nakayama T, Hiyama Y, Iwama S, Goto N, Nakajima Y, Musha N, Nakashima T. Formation of hilar collaterals or cavernous transformation after portal vein obstruction by hepatocellular carcinoma. Observations in ten patients. *Gastroenterology* 1984; **87**: 1150-1153 [PMID: 6090259]

2 **Valla DC**, Condat B. Portal vein thrombosis in adults: pathophysiology, pathogenesis and management. *J Hepatol* 2000; **32**: 865-871 [PMID: 10845677]

3 **Ponziani FR**, Zocco MA, Campanale C, Rinninella E, Tortora A, Di Maurizio L, Bombardieri G, De Cristofaro R, De Gaetano AM, Landolfi R, Gasbarrini A. Portal vein thrombosis: insight into physiopathology, diagnosis, and treatment. *World J Gastroenterol* 2010; **16**: 143-155 [PMID: 20066733]

4 **DeLeve LD**, Valla DC, Garcia-Tsao G. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]

5 **de Franchis R**. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742]

6 **De Stefano V**, Martinelli I. Splanchnic vein thrombosis: clinical presentation, risk factors and treatment. *Intern Emerg Med* 2010; **5**: 487-494 [PMID: 20532730 DOI: 10.1007/s11739-010-0413-6]

7 **Rajani R**, Björnsson E, Bergquist A, Danielsson A, Gustavsson A, Grip O, Melin T, Sangfelt P, Wallerstedt S, Almer S. The epidemiology and clinical features of portal vein thrombosis: a multicentre study. *Aliment Pharmacol Ther* 2010; **32**: 1154-1162 [PMID: 21039677 DOI: 10.1111/j.1365-2036.2010.04454.x]

8 **Janssen HL**, Wijnhoud A, Haagsma EB, van Uum SH, van Nieuwkerk CM, Adang RP, Chamuleau RA, van Hattum J, Vleggaar FP, Hansen BE, Rosendaal FR, van Hoek B. Extrahepatic portal vein thrombosis: aetiology and determinants of survival. *Gut* 2001; **49**: 720-724 [PMID: 11600478]

9 **Orr DW**, Harrison PM, Devlin J, Karani JB, Kane PA, Heaton ND, O'Grady JG, Heneghan MA. Chronic mesenteric venous thrombosis: evaluation and determinants of survival during long-term follow-up. *Clin Gastroenterol Hepatol* 2007; **5**: 80-86 [PMID: 17142105]

10 **Qi X**, Han G, Yin Z, He C, Guo W, Niu J, Wu K, Fan D. Cavernous vessels around a patent portal trunk in the liver hilum. *Abdom Imaging* 2012; **37**: 422-430 [PMID: 21789553 DOI: 10.1007/s00261-011-9779-9]

11 **Qi X**, Han G, Yin Z, He C, Bai M, Yang Z, Guo W, Niu J, Wu K, Fan D. A large portal vein: a rare finding of recent portal vein thrombosis. *Case Rep Gastroenterol* 2011; **5**: 33-39 [PMID: 21326856 DOI: 10.1159/000323386]

12 **Qi X**, Han G, Yin Z, He C, Wang J, Guo W, Niu J, Zhang W, Bai M, Fan D. Transjugular intrahepatic portosystemic shunt for portal cavernoma with symptomatic portal hypertension in non-cirrhotic patients. *Dig Dis Sci* 2012; **57**: 1072-1082 [PMID: 22147244 DOI: 10.1007/s10620-011-1975-5]

13 **Qi X**, He C, Han G, Yin Z, Wu F, Zhang Q, Niu J, Wu K, Fan D. Prevalence of paroxysmal nocturnal hemoglobinuria in

Chinese patients with Budd-Chiari syndrome or portal vein thrombosis. *J Gastroenterol Hepatol* 2013; **28**: 148-152 [PMID: 23020629 DOI: 10.1111/j.1440-1746.2012.07282.x]

14 **Qi X**, Zhang C, Han G, Zhang W, He C, Yin Z, Liu Z, Bai W, Li R, Bai M, Yang Z, Wu K, Fan D. Prevalence of the JAK2V617F mutation in Chinese patients with Budd-Chiari syndrome and portal vein thrombosis: a prospective study. *J Gastroenterol Hepatol* 2012; **27**: 1036-1043 [PMID: 22142461 DOI: 10.1111/j.1440-1746.2011.07040.x]

15 **Qi X**, Han G, He C, Yin Z, Guo W, Niu J, Fan D. CT features of non-malignant portal vein thrombosis: a pictorial review. *Clin Res Hepatol Gastroenterol* 2012; **36**: 561-568 [PMID: 22883835]

16 **Qi X**, Han G, Bai M, Fan D. Stage of portal vein thrombosis. *J Hepatol* 2011; **54**: 1080-102; author reply 1080-102 [PMID: 21145872]

17 **Qi X**, Han G, Wang J, Wu K, Fan D. Degree of portal vein thrombosis. *Hepatology* 2010; **51**: 1089-1090 [PMID: 19957371 DOI: 10.1002/hep.23397]

18 **Han G**, Qi X, He C, Yin Z, Wang J, Xia J, Yang Z, Bai M, Meng X, Niu J, Wu K, Fan D. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with symptomatic portal hypertension in liver cirrhosis. *J Hepatol* 2011; **54**: 78-88 [PMID: 20932597]

19 **Idezuki Y**. General rules for recording endoscopic findings of esophagogastric varices (1991). Japanese Society for Portal Hypertension. *World J Surg* 1995; **19**: 420-422; discussion 423 [PMID: 7638999]

20 **Moore KP**, Wong F, Gines P, Bernardi M, Ochs A, Salerno F, Angeli P, Porayko M, Moreau R, Garcia-Tsao G, Jimenez W, Planas R, Arroyo V. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. *Hepatology* 2003; **38**: 258-266 [PMID: 12830009 DOI: 10.1053/jhep.2003.50315]

21 **Qi X**, Han G, Wu K, Fan D. Anticoagulation for portal vein thrombosis in cirrhosis. *Am J Med* 2010; **123**: e19-20; author reply e21 [PMID: 20800136]

22 **Qi X**, Han G. Transjugular intrahepatic portosystemic shunt in the treatment of portal vein thrombosis: a critical review of literature. *Hepatol Int* 2012; **6**: 576-590 [DOI: 10.1007/s12072-011-9324-5]

23 **Amitrano L**, Guardascione MA, Scaglione M, Pezzullo L, Sangiuliano N, Armellino MF, Manguso F, Margaglione M, Ames PR, Iannaccone L, Grandone E, Romano L, Balzano A. Prognostic factors in noncirrhotic patients with splanchnic vein thromboses. *Am J Gastroenterol* 2007; **102**: 2464-2470 [PMID: 17958760]

24 **Webb LJ**, Sherlock S. The aetiology, presentation and natural history of extra-hepatic portal venous obstruction. *Q J Med* 1979; **48**: 627-639 [PMID: 538223]

25 **Spaander MC**, van Buuren HR, Hansen BE, Janssen HL. Ascites in patients with noncirrhotic nonmalignant extrahepatic portal vein thrombosis. *Aliment Pharmacol Ther* 2010; **32**: 529-534 [PMID: 20497136]

26 **Qi X**, De Stefano V, Wang J, Bai M, Yang Z, Han G, Fan D. Prevalence of inherited antithrombin, protein C, and protein S deficiencies in portal vein system thrombosis and Budd-Chiari syndrome: a systematic review and meta-analysis of observational studies. *J Gastroenterol Hepatol* 2013; **28**: 432-442 [PMID: 23216127 DOI: 10.1111/jgh.12085]

27 **Qi X**, Yang Z, Bai M, Shi X, Han G, Fan D. Meta-analysis: the significance of screening for JAK2V617F mutation in Budd-Chiari syndrome and portal venous system thrombosis. *Aliment Pharmacol Ther* 2011; **33**: 1087-1103 [PMID: 21395632 DOI: 10.1111/j.1365-2036.2011.04627.x]

P- Reviewers: Castellote J, Raziorrouh B **S- Editor:** Zhai HH
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doi:10.3748/wjg.v19.i42.7455

World J Gastroenterol 2013 November 14; 19(42): 7455-7460
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

Effect of low-dose amitriptyline on globus pharyngeus and its side effects

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Received: July 22, 2013 **Revised:** September 26, 2013
Accepted: September 29, 2013
Published online: November 14, 2013

Abstract

AIM: To compare the efficacy and side effects of low-dose amitriptyline (AMT) with proton pump inhibitor treatment in patients with globus pharyngeus.

METHODS: Thirty-four patients who fulfilled the Rome Ⅲ criteria for functional esophageal disorders were included in this study. Patients were randomly assigned to receive either 25 mg AMT before bedtime (AMT group) or 40 mg Pantoprazole once daily for 4 wk (conventional group). The main efficacy endpoint was assessed using the Glasgow Edinburgh Throat Scale (GETS). The secondary efficacy endpoints included the Medical Outcomes Study 36-item short form health survey [social functioning (SF)-36] and the Pittsburgh Sleep Quality Index. Treatment response was defined as a > 50% reduction in GETS scores. All patients entering this study recorded side effects at days 1, 8, 15, 22 and 29 using a visual analogue scale.

RESULTS: Thirty patients completed the study. After 4 wk of treatment, the AMT group had a greater response than the conventional group (75% *vs* 35.7%, *P* = 0.004). At day 3, the AMT group showed significantly more improvement than the Conventional group in GETS score (3.69 ± 1.14 *vs* 5.64 ± 1.28, *P* = 0.000). After 4 wk of treatment, the AMT group showed significantly greater improvement in GETS score and sleep quality than the Conventional group (1.25 ± 1.84 *vs* 3.79 ± 2.33, 4.19 ± 2.07 *vs* 8.5 ± 4.97; *P* < 0.01 for both). Additionally, the AMT group was more likely than the Conventional group to experience improvement in the SF-36, including general health, vitality, social functioning and mental health (*P* = 0.044, 0.024, 0.049 and 0.005). Dry mouth, sleepiness, dizziness and constipation were the most common side effects.

CONCLUSION: Low-dose AMT is well tolerated and can significantly improve patient symptoms, sleep and quality of life. Thus, low-dose AMT may be an effective treatment for globus pharyngeus.

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Key words: Amitriptyline; Globus pharyngeus; Side effect; Pantoprazole; Treatment response

Core tip: A literature review reveals that there is no single effective treatment for patients with globus pharyngeus. Low-dose amitriptyline (AMT) is extensively used to treat functional gastrointestinal disorders, especially in cases with prolonged severe symptoms and disorders that affect daily function. However, no data regarding the possible effects of AMT in patients with globus pharyngeus are available. In this study, we conclude that low-dose AMT is well tolerated and can significantly improve patient symptoms. Thus, we recommend the use of low-dose AMT for globus pharyngeus.

You LQ, Liu J, Jia L, Jiang SM, Wang GQ. Effect of low-dose amitriptyline on globus pharyngeus and its side-effects. *World J*

Gastroenterol 2013; 19(42): 7455-7460 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7455.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7455

INTRODUCTION

Globus pharyngeus is a condition characterized by a nonpainful sensation of a lump in the throat in the absence of true dysphagia or odynophagia; the sensation frequently improves with eating[1]. It is a common condition that accounts for approximately 4% of otolaryngological referrals[2], and it is usually long-lasting, difficult to treat, recurrent and associated with a significant impairment in quality of life. Furthermore, due to the uncertain etiology of globus, it remains difficult to establish standard investigation and treatment strategies for affected patients.

Amitriptyline (AMT) is a tricyclic antidepressant with limited application due to the side effects caused by high doses (100 mg/d). In recent years, low-dose AMT has been shown to be well tolerated and significantly effective in improving the functional gastrointestinal disorders[3-5]. In 1994, Deary *et al*[6] were the first to attempt a prospective, randomized, double-blinded, placebo-controlled trial investigating the effectiveness of AMT in patients with globus pharyngeus. Most patients could not tolerate the side effects of AMT at doses of 50 mg/d to 150 mg/d, resulting in treatment failure. To the best of our knowledge, evidence supporting the possible effects of low-dose AMT in patients with globus pharyngeus has not been reported.

Therefore, the aim of this study was to investigate the response rate, onset time, side effects and clinical predictors of symptom response to low-dose AMT treatment in patients with globus pharyngeus.

MATERIALS AND METHODS

Patients

In this prospective study, we enrolled 34 patients who complained of globus symptoms between September 2011 and January 2013. All patients were between 12 and 65 years of age and were newly diagnosed as having functional esophageal disorders based on the following Rome Ⅲ criteria[7]: (1) persistent or intermittent, nonpainful sensation of a lump or foreign body in the throat; (2) occurrence of the sensation between meals; (3) absence of dysphagia or odynophagia; (4) absence of evidence that gastroesophageal reflux is the cause of the symptom; and (5) absence of histopathology-based diagnosis of esophageal motility disorders. All included patients fulfilled the criteria for the last 3 mo, with symptom onset at least 6 mo before diagnosis. All patients underwent otolaryngological assessment with neck/thyroid palpation and gastroscopy or laryngoscopy, and none had any organic abnormality on assessment. The following exclusion criteria were adopted: hepatic or renal disease; prostatic disease; pregnancy or breast feeding; known glaucoma; history of seizures; history of thyroid or liver dysfunction; recent use of monoamine oxidase inhibitors; use of any proton pump inhibitor (PPI) or histamine type 2 receptor antagonist during the last 2 mo; use of tranquilizers or antidepressants that may affect esophageal motor function; and moderate to severe anxiety or depression (14-item Hamilton Anxiety Rating Scale assessed from 0 to 13 points and 17-item Hamilton Depression Rating Scale assessed from 0 to 17 points).

Study design and procedures

This study was a prospective, randomized controlled trial in globus pharyngeus patients and was approved by the hospital ethics committee (Clinical trial registration number: ChiCTR-TRC-12001968). Written informed consent was obtained from the patients according to the Declaration of Helsinki.

Thirty-four eligible patients were randomized to receive either 25 mg AMT once daily before bedtime or 40 mg Pantoprazole once daily for 4 wk. Treatment was allocated by a simple randomization method using a computer-generated randomization schedule. Ultimately, 17 patients received AMT, and 17 patients received Pantoprazole. The primary endpoint was assessed using the Glasgow Edinburgh Throat Scale (GETS) questionnaire[8]. We observed the onset time and the treatment efficiency on days 3 and 10 and week 4, and evaluated the social functioning (SF)-36 and Pittsburgh sleep quality index (PSQI) scores at baseline and at week 4 as secondary endpoints[9,10].

All patients entering this study recorded side effects. The severity of side effects was evaluated using a visual analogue scale (VAS) administered on days 1, 8, 15, 22 and 29 after medication.

GETS

The GETS questionnaire provided both the primary and secondary outcomes. It is a validated questionnaire used to rate globus pharyngeus symptoms. The globus symptom score component is based on 10 questions assessing various throat symptoms. Patients subjectively grade their symptoms for each question on a 7-point Likert scale, with 0 being “none” and 7 being “unbearable”. These compiled questions yield a score that represents the severity of the patient’s globus symptoms, with a maximum possible score of 70. The secondary outcome is the somatic distress score, which represents the psychological impact of the patient’s symptoms. This component of the questionnaire is also graded on a 7-point Likert scale, with 0 being “never” and 7 being “all of the time”. This yields a maximum total score of 14. Both the overall symptom score and the somatic distress score can be used over time to assess the severity of the disease.

Medical outcomes study 36-item short form health survey

The SF-36 is the most commonly used scale for assessing patient quality of life, and it includes eight dimensions:

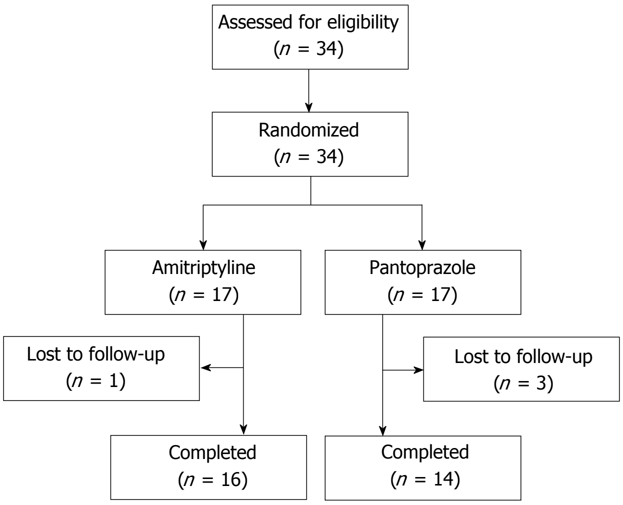

Figure 1 Consort diagram.

Table 1 Demographic and baseline characteristics of the study patients

Variable	AMT group *n* = 16	Conventional group *n* = 14	*P* value
Age (yr)	43.19 ± 10.96	42.93 ± 13.1	0.953
Gender (male/female)	6/10	5/9	1.000
BMI	21.54 ± 2.09	23.01 ± 3.49	0.166
Smoking habit	4 (18.75%)	3 (21.42%)	1.000
Alcohol consumption	6 (37.5%)	5 (35.71%)	1.000
Symptom duration (yr)	1.44 ± 0.66	1.54 ± 0.89	0.731
GETS	5.44 ± 1.63	5.71 ± 1.38	0.623
PSQI	8.75 ± 4.68	8.71 ± 5.27	0.984

Values are represented as the mean ± SD. AMT: Amitriptyline; BMI: Body mass index; GETS: Glasgow Edinburgh Throat Scale; PSQI: Pittsburgh sleep quality index.

physical functioning, role-physical (RP), bodily pain, general health (GH), vitality (VT), SF, role-emotional (RE), and mental health (MH). A higher score indicates a better quality of life.

PSQI

Prepared by psychiatrist Buysse *et al*[9], the PSQI assesses seven areas, including sleep quality, the time to fall asleep, sleep time, sleep efficiency, sleep disturbance, use of sleep medication and daytime dysfunction. In total, 18 items were used for the calculation of the PSQI score. Higher scores represent worse sleep quality, and a score > 7 points indicates the presence of a sleep disorder.

Treatment response

Treatment response was defined as a > 50% reduction in the GETS score. The response was calculated as: [(score at treatment - score at baseline)/score at baseline] × 100. The treatment responses of the two groups were calculated separately.

Statistical analysis

Data analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago IL, United States), and the measurement data are reported as the mean ± SD; baseline parameters and differences between the two treatments were compared using Student's *t* test. The scores at baseline and after AMT or Pantoprazole treatment were compared by paired *t* test. The VAS scores of side effects were analyzed using the χ^2 test or Fisher's exact test. *P* < 0.05 was considered statistically significant.

Table 2 Change in glasgow edinburgh throat scale score and Pittsburgh sleep quality index in patients treated with amitriptyline *vs* pantoprazole and incidence of side effects *n* (%)

Variable	AMT group *n* = 16	Conventional group *n* = 14	*P* value
GETS score			
Baseline	5.44 ± 1.63	5.71 ± 1.38	0.623
3 d	3.69 ± 1.14^1	5.64 ± 1.28	0.000
10 d	2 ± 1.71^1	5.36 ± 1.22	0.000
4 wk	1.25 ± 1.84^1	3.79 ± 2.33^1	0.002
Treatment response	12 (75)	5 (35.71)	0.004
PSQI	4.19 ± 2.07	8.5 ± 4.97	0.008
Adverse effect			
Dry mouth	12 (75)	4 (28.5)	0.026
Sleepiness	11 (68.8)	2 (14.3)	0.004
Dizziness	4 (25)	1 (7.1)	0.336
Constipation	3 (18.8)	1 (7.1)	0.602
Palpitations	1 (6.3)	0 (0)	1.000
Malaise	1 (6.3)	0 (0)	1.000

[1] Statistically significant difference from baseline, *P* < 0.05 between the two groups. AMT: Amitriptyline; GETS: Glasgow Edinburgh Throat Scale; PSQI: Pittsburgh sleep quality index.

RESULTS

Study participants

A total of 34 patients with globus symptom were enrolled in the study. All patients were randomized to receive either AMT or Pantoprazole. Of these patients, four were lost to follow-up. A total of 30 patients (AMT 16, Pantoprazole 14) completed the study (Figure 1). The baseline characteristics of the patients are shown in Table 1. No differences were observed between the two groups in age, gender, body mass index, smoking habit, alcohol consumption, symptom duration, GETS score or PSQI score.

Primary efficacy endpoint

The rate of effectiveness was calculated in the two groups after 4 wk of treatment, the response rate of the AMT group was significantly higher than that of the Conventional group (75% *vs* 35.7%, *P* = 0.004, Table 2). Compared with the Conventional group, the GETS scores of the AMT group were significantly improved at days 3 and 10, and week 4 (all *P* < 0.01, Table 2). Compared with baseline, the GETS scores of the AMT group were significantly reduced at days 3 and 10 and week 4 (all *P* < 0.05). However, the GETS scores of the Conventional group were significantly reduced only after 4 wk of treatment. The onset time in the AMT group was significantly earlier than that in the Conventional group.

Table 3 Changes in social functioning-36 subscale scores in patients treated with amitriptyline *vs* Pantoprazole

Variable		Baseline	Week 4	*P* value
PF	AMT	95 ± 11.4	95.94 ± 11.14	0.945
	Conventional	93.93 ± 7.12	95.71 ± 4.75	
RP	AMT	64.06 ± 37.6	81.25 ± 26.61[1]	0.929
	Conventional	66.07 ± 37.48	80.36 ± 28.04[1]	
BP	AMT	89.38 ± 16.52	90.63 ± 14.55	0.498
	Conventional	85.57 ± 20.68	86.29 ± 19.96	
GH	AMT	56.75 ± 20.92	73 ± 17.57[1]	0.044
	Conventional	49.86 ± 22.49	58.71 ± 19.57[1]	
VT	AMT	70.63 ± 18.15	88.44 ± 7.01[1]	0.024
	Conventional	71.79 ± 24.7	73.21 ± 24.39	
SF	AMT	75 ± 18.25	92.97 ± 13.67[1]	0.049
	Conventional	77.68 ± 18.46	81.25 ± 17.51[1]	
RE	AMT	68.76 ± 35.42	79.18 ± 23.96[1]	0.384
	Conventional	61.91 ± 41.05	69.05 ± 38.04	
MH	AMT	68.75 ± 15.68	83.5 ± 10[1]	0.005
	Conventional	65.43 ± 19.32	69.14 ± 15.72	

[1] Statistically significant difference compared with baseline, $P < 0.05$ between the two groups. AMT: Amitriptyline; PF: Physical functioning; RP: Role-physical; BP: Bodily pain; GH: General health; VT: Vitality; SF: Social functioning; RE: Role-emotional; MH: Mental health.

Secondary efficacy endpoints

Compared with the Conventional group, the AMT group exhibited significant improvements in the GH, VT, SF, MH scales of the SF-36 (all $P < 0.05$, Table 3). Compared with baseline, the AMT group exhibited significant improvements in RP, GH, VT, SF, RE and MH (all $P < 0.05$). The Conventional group experienced significant improvements in only RP, GH and SF (all $P < 0.05$, Table 3).

The AMT group also showed a significant improvement in PSQI compared with the Conventional group (4.19 ± 2.07 *vs* 8.5 ± 4.97, $P = 0.008$, Table 2). Compared with baseline, the AMT group exhibited significant improvement. However, there were no significant differences in PSQI at baseline and after pantoprazole treatment ($P > 0.05$).

Adverse effects

The various adverse events reported in the two treatment groups are shown in Table 2. The incidence rates of dry mouth and sleepiness in the AMT group were significantly higher than in the Conventional group ($P = 0.026$, $P = 0.004$, Table 2). The incidence rates of dizziness and constipation were also somewhat higher in the AMT group than in the Conventional group, although the difference was not statistically significant ($P = 0.336$, $P = 0.602$, Table 2).

As shown in Figure 2, sleepiness and dizziness almost disappeared within 1 wk. Patients who had dry mouth and constipation symptoms experienced significant relief after receiving AMT for 2 wk. No serious adverse events occurred in either group.

DISCUSSION

There is no consistent evidence attributing globus to any

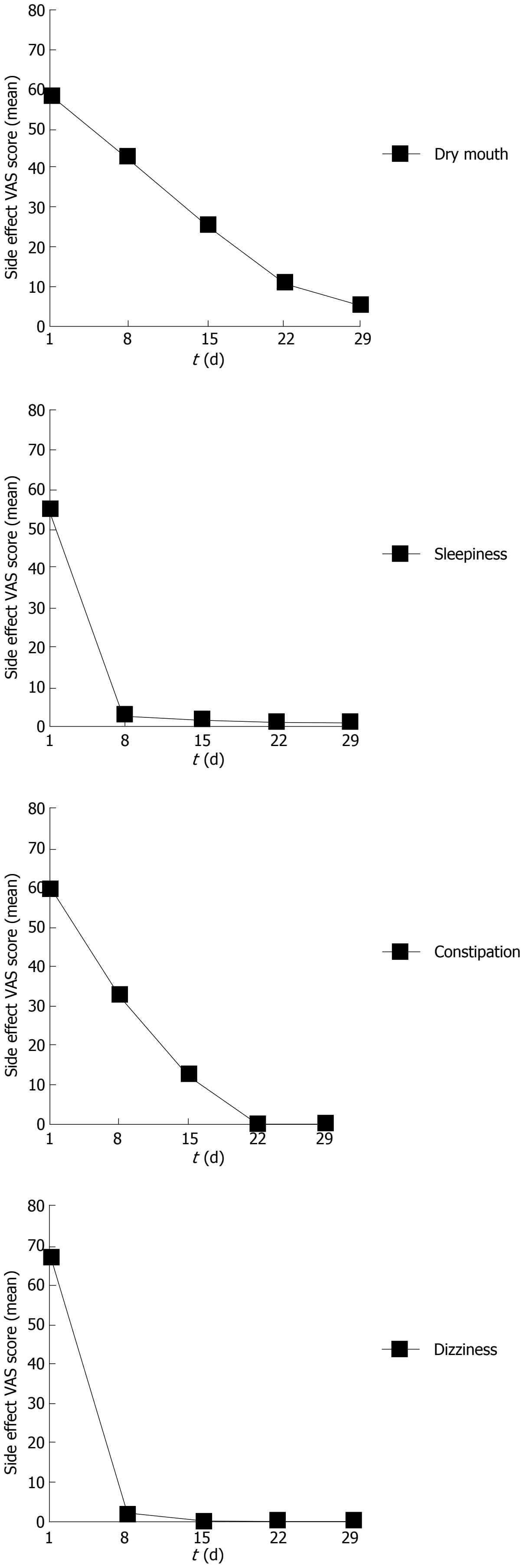

Figure 2 Side effect diagrams. VAS: Visual analogue scale.

specific anatomic abnormality, including the cricopharyngeal bar. Although it has been long since it was first described, the etiology of globus pharyngeus is poorly understood. Abnormal upper esophageal sphincter function, esophageal motility disorders, psychological factors, stress, and *Helicobacter pylori* infection of the cervical heterotopic gastric mucosa have all been suggested as potential causes of globus[11-15]. There is much controversy about the true etiology of globus pharyngeus, and it has been difficult to establish a causal relationship between globus and these other disorders[16]. In addition, esophageal balloon distention can simulate the sensation of globus at low distending thresholds, suggesting some degree of esophageal hypersensitivity[1]. Because there are few controlled studies on the treatment of globus, evidence-based treatment for this disorder is currently not available. Some studies have suggested that gastroesophageal reflux disease (GERD) is a major cause of globus[16,17-19]. Therefore, it seems practical to use anti-reflux methods as the first-line treatment for managing patients with globus[11], although this protocol remains under considerable debate. Other established treatment options include speech and language therapy, anti-depressants, and cognitive-behavioral therapy[11,20-22].

Low-dose AMT has been used widely in gastroenterologic practice. Morgan *et al*[23] demonstrated that the doses for the analgesic and neuromodulatory effects of AMT were below the effective doses of the antidepressant. Huang *et al*[24] observed that low-dose AMT could reduce visceral sensitivity using the noninvasive drinking-ultrasonography test in healthy volunteers. However, our understanding of the mechanism of action of this agent is limited. A commonly held hypothesis is that AMT, which indirectly stimulates norepinephrine and serotonin receptors by blocking the reuptake of norepinephrine and serotonin, works as a neuromodulator, affecting the brain-gut axis by altering neurotransmitter systems within the limbic system and other pain centers of the brain[25]. In addition, one study showed that AMT can alter sleep patterns[25]. Our study also confirmed that the AMT group showed significantly greater improvement in sleep than the Conventional group. We hypothesize that the overall effects of amitriptyline in patients with globus arise from its central nervous system activity, as altering sleep patterns modulates the regulation of the noradrenergic system of the locus coeruleus (a brain center inhibited during sleep), which alters nociception.

In our study, 5 of 14 (35.7%) patients with globus pharyngeus receiving PPI treatment as a conventional therapy were classified as responders. Dumper *et al*[26] demonstrated that there was no clinically or statistically significant difference between lansoprazole and placebo at any time point during a 3-mo treatment period. Therefore, it is important to note that although GERD is a condition that is sometimes associated with globus, this does not mean that all globus is GERD-related or that all GERD patients have globus. Thus, PPI treatment in patients with globus may not achieve satisfactory results.

To our knowledge, this is the first prospective, randomized controlled trial investigating the effectiveness of a therapeutic low dose of AMT in globus patients. The treatment efficiency of the AMT group was 75%, significantly greater than that of the Conventional group (35.7%). After 3 d of treatment, the symptoms, sleep, and quality of life of the AMT group improved more than those of the Conventional group. During treatment, average cost in the AMT group was also significantly lower than that in the Conventional group.

There are several limitations of this study. First, although many patients with globus symptoms and normal examinations were seen in our clinic, this study included only a small sample of patients with globus. A number of patients had recently started on PPI or were diagnosed with moderate to severe anxiety or depression, and excluding these patients resulted in a decreased number of cases in this study. Second, it is possible that the course of medication in our study was too short; the standard duration of AMT administration in the clinic is 4-12 wk[27]. We also evaluated only the response to short-term PPI treatment and did not investigate the response to long-term PPI treatment (3 or more months).

In conclusion, low-dose AMT is well tolerated and may be effective in reducing the symptoms of patients with globus pharyngeus while also significantly improving sleep and quality of life. However, further studies with larger sample sizes and longer follow-up periods are needed to verify our results.

ACKNOWLEDGMENTS

We would like to thank Ming-Zhi Xu, Professor of the Guangdong Mental Disorder Research Institute, for his guidance in the use of SF-36 and the anxiety and depression scale. We also express our appreciation to Dr. Su of ENT for his assistance in diagnosing globus pharyngeus.

COMMENTS

Background

Low-dose amitriptyline (AMT) is extensively used for treating functional gastrointestinal disorders, although its precise mechanism of action is still not fully understood. In 1994, Deary *et al* performed the first study investigating the effectiveness of high-dose AMT (50-150 mg/d) in patients with globus pharyngeus. However, most patients could not tolerate the side effects of high doses of AMT, resulting in treatment failure. The effect of low-dose AMT in patients with globus pharyngeus has not been reported.

Research frontiers

There is currently a lack of highly effective pharmacotherapy for globus pharyngeus. Low-dose AMT may be a good choice of treatment for patients with globus pharyngeus.

Innovations and breakthroughs

This is the first prospective, randomized controlled trial evaluating the effectiveness of a therapeutic low dose of AMT in globus patients. The results revealed that low-dose AMT is well tolerated and can significantly improve patient symptoms, sleep and quality of life.

Applications

Low-dose AMT can be significantly effective in improving the symptoms and quality of life of the patients, thereby supporting its clinical applicability for gastrointestinal disorders.

Terminology

Globus pharyngeus is a physical sensation of a lump in the throat that causes difficulty or discomfort in swallowing. The sensation may also be choking or feeling as though there is a mass lodged in the esophagus. AMT is a tricyclic antidepressant that is extensively used to treat functional gastrointestinal diseases, especially in cases with prolonged severe symptoms and daily functional disorders.

Peer review

This is an interesting paper evaluating the effect of low-dose AMT on globus pharyngeus. The study is reasonably well done, and the design and outcome assessment are standardized.

REFERENCES

1 **Galmiche JP**, Clouse RE, Bálint A, Cook IJ, Kahrilas PJ, Paterson WG, Smout AJ. Functional esophageal disorders. *Gastroenterology* 2006; **130**: 1459-1465 [PMID: 16678559 DOI: 10.1053/j.gastro.2005.08.060]

2 **Moloy PJ**, Charter R. The globus symptom. Incidence, therapeutic response, and age and sex relationships. *Arch Otolaryngol* 1982; **108**: 740-744 [PMID: 7138370]

3 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. Efficacy of tricyclic antidepressants in irritable bowel syndrome: a meta-analysis. *World J Gastroenterol* 2009; **15**: 1548-1553 [PMID: 19340896]

4 **Teitelbaum JE**, Arora R. Long-term efficacy of low-dose tricyclic antidepressants for children with functional gastrointestinal disorders. *J Pediatr Gastroenterol Nutr* 2011; **53**: 260-264 [PMID: 21865971 DOI: 10.1097/MPG.0b013e318217df7c]

5 **Thoua NM**, Murray CD, Winchester WJ, Roy AJ, Pitcher MC, Kamm MA, Emmanuel AV. Amitriptyline modifies the visceral hypersensitivity response to acute stress in the irritable bowel syndrome. *Aliment Pharmacol Ther* 2009; **29**: 552-560 [PMID: 19076934 DOI: 10.1111/j.1365-2036.2008.03918]

6 **Deary IJ**, Wilson JA. Problems in treating globus pharyngis. *Clin Otolaryngol Allied Sci* 1994; **19**: 55-60 [PMID: 8174303]

7 **Drossman DA**. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology* 2006; **130**: 1377-1390 [PMID: 16678553]

8 **Deary IJ**, Wilson JA, Harris MB, MacDougall G. Globus pharyngis: development of a symptom assessment scale. *J Psychosom Res* 1995; **39**: 203-213 [PMID: 7595878]

9 **Buysse DJ**, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989; **28**: 193-213 [PMID: 2748771]

10 **Ware JE**, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; **30**: 473-483 [PMID: 1593914]

11 **Lee BE**, Kim GH. Globus pharyngeus: a review of its etiology, diagnosis and treatment. *World J Gastroenterol* 2012; **18**: 2462-2471 [PMID: 22654443 DOI: 10.3748/wjg.v18.i20.2462]

12 **Kwiatek MA**, Mirza F, Kahrilas PJ, Pandolfino JE. Hyperdynamic upper esophageal sphincter pressure: a manometric observation in patients reporting globus sensation. *Am J Gastroenterol* 2009; **104**: 289-298 [PMID: 19174789 DOI: 10.1038/ajg.2008.150]

13 **Färkkilä MA**, Ertama L, Katila H, Kuusi K, Paavolainen M, Varis K. Globus pharyngis, commonly associated with esophageal motility disorders. *Am J Gastroenterol* 1994; **89**: 503-508 [PMID: 8147350]

14 **Harris MB**, Deary IJ, Wilson JA. Life events and difficulties in relation to the onset of globus pharyngis. *J Psychosom Res* 1996; **40**: 603-615 [PMID: 8843039]

15 **Alagozlu H**, Simsek Z, Unal S, Cindoruk M, Dumlu S, Dursun A. Is there an association between Helicobacter pylori in the inlet patch and globus sensation? *World J Gastroenterol* 2010; **16**: 42-47 [PMID: 20039447]

16 **Tsutsui H**, Manabe N, Uno M, Imamura H, Kamada T, Kusunoki H, Shiotani A, Hata J, Harada T, Haruma K. Esophageal motor dysfunction plays a key role in GERD with globus sensation--analysis of factors promoting resistance to PPI therapy. *Scand J Gastroenterol* 2012; **47**: 893-899 [PMID: 22594305 DOI: 10.3109/00365521.2012.685756]

17 **Tokashiki R**, Yamaguchi H, Nakamura K, Suzuki M. Globus sensation caused by gastroesophageal reflux disease. *Auris Nasus Larynx* 2002; **29**: 347-351 [PMID: 12393039]

18 **Chevalier JM**, Brossard E, Monnier P. Globus sensation and gastroesophageal reflux. *Eur Arch Otorhinolaryngol* 2003; **260**: 273-276 [PMID: 12750918]

19 **Tokashiki R**, Funato N, Suzuki M. Globus sensation and increased upper esophageal sphincter pressure with distal esophageal acid perfusion. *Eur Arch Otorhinolaryngol* 2010; **267**: 737-741 [PMID: 19882344 DOI: 10.1007/s00405-009-1134-1]

20 **Khalil HS**, Bridger MW, Hilton-Pierce M, Vincent J. The use of speech therapy in the treatment of globus pharyngeus patients. A randomised controlled trial. *Rev Laryngol Otol Rhinol* (Bord) 2003; **124**: 187-190 [PMID: 14725135]

21 **Cybulska EM**. Globus hystericus--a somatic symptom of depression? The role of electroconvulsive therapy and antidepressants. *Psychosom Med* 1997; **59**: 67-69 [PMID: 9021868]

22 **Burns P**, O'Neill JP. The diagnosis and management of globus: a perspective from Ireland. *Curr Opin Otolaryngol Head Neck Surg* 2008; **16**: 503-506 [PMID: 19005320 DOI: 10.1097/MOO.0b013e3283169258]

23 **Morgan V**, Pickens D, Gautam S, Kessler R, Mertz H. Amitriptyline reduces rectal pain related activation of the anterior cingulate cortex in patients with irritable bowel syndrome. *Gut* 2005; **54**: 601-607 [PMID: 15831901 DOI: 10.1136/gut.2004.047423]

24 **Huang W**, Jiang SM, Jia L, You LQ, Huang YX, Gong YM, Wang GQ. Effect of amitriptyline on gastrointestinal function and brain-gut peptides: a double-blind trial. *World J Gastroenterol* 2013; **19**: 4214-4220 [PMID: 23864786 DOI: 10.3748/wjg.v19.i26.4214]

25 **Mertz H**, Fass R, Kodner A, Yan-Go F, Fullerton S, Mayer EA. Effect of amitriptyline on symptoms, sleep, and visceral perception in patients with functional dyspepsia. *Am J Gastroenterol* 1998; **93**: 160-165 [PMID: 9468233]

26 **Dumper J**, Mechor B, Chau J, Allegretto M. Lansoprazole in globus pharyngeus: double-blind, randomized, placebo-controlled trial. *J Otolaryngol Head Neck Surg* 2008; **37**: 657-663 [PMID: 19128672 DOI: 10.2310/7070.2008.0107]

27 **Rajagopalan M**, Kurian G, John J. Symptom relief with amitriptyline in the irritable bowel syndrome. *J Gastroenterol Hepatol* 1998; **13**: 738-741 [PMID: 9715427]

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Gastroenterology

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doi:10.3748/wjg.v19.i42.7461

World J Gastroenterol 2013 November 14; 19(42): 7461-7471
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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META-ANALYSIS

Combined radiochemotherapy in patients with locally advanced pancreatic cancer: A meta-analysis

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Author contributions: All the authors were involved in the design of the protocol and assessment of the studies identified; Chen Y and Sun XJ performed data entry and analysis; Jiang TH, Mao AW and Chen Y drafted the manuscript; all authors have read and approved the final version to be published.
Supported by Shanghai Science and Technology Development Fund, No. 10JC1412902
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Received: June 2, 2013 **Revised:** August 8, 2013
Accepted: August 20, 2013
Published online: November 14, 2013

Abstract

AIM: To compare the long-term clinical efficacy of chemotherapy plus radiotherapy (CRT) with that of radiotherapy alone (RT) or chemotherapy alone (CT) for locally advanced pancreatic carcinoma (LAPC).

METHODS: Using manual and computer-aided methods, we searched the data through the databases, including PubMed/EmBase/CNKI/CQVIP/China Journals Full Text Database and websites and proceedings of major annual meetings such as ASCO and CSCO. The methodological quality of the included studies was assessed using the Jadad scoring system. Both English and Chinese publications were searched. We collected data from controlled clinical trials on CRT *vs* RT or CT for LAPC, and conducted a meta-analysis of 15 included studies. Meta-analysis was performed using RevMan4.2 Software according to the method recommended by Cochrane Collaboration.

RESULTS: Fifteen eligible randomized controlled trials including a total of 1128 patients were screened. Jadad score was 2 in only one article, and 3-4 in the remaining 14 studies. The meta-analysis showed that CRT was superior in the 6- and 12-mo survivals to the RT alone group or CT alone group (P = 0.0001 and P = 0.02, respectively), whereas the 18-mo survival showed no significant difference (P = 0.23). Subgroup analysis showed that the 6-, 12-, and 18-mo survivals were not significantly different between the CRT group and CT group (P = 0.07, P = 0.23, and P = 0.91, respectively). Notably, the CRT group had significantly better 6-, 12-, and 18-mo survivals than the RT group (all $P < 0.01$). CRT group had significantly more grade 3-4 treatment-related hematologic and non-hematologic toxicities than the CT group or RT group (all $P < 0.01$).

CONCLUSION: Compared with CT or RT, CRT can benefit the long-term survival of LAPC patients, although it may also increase treatment-related toxicities.

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Key words: Pancreatic cancer; Chemotherapy; Radiotherapy; Meta-analysis; Survival

Core tip: To compare the long-term clinical efficacy of chemotherapy plus radiotherapy (CRT) with that of radiotherapy alone (RT) or chemotherapy alone (CT) for locally advanced pancreatic carcinoma (LAPC)，the authors analyzed the potential impact of CRT, CT or RT on the survival of the patients using meta-analysis methodologies. Meta-analysis showed that compared with CT or RT, CRT can benefit the long-term survival of LAPC patients, although it may also increase treatment-related toxicities.

Chen Y, Sun XJ, Jiang TH, Mao AW. Combined radiochemotherapy in patients with locally advanced pancreatic cancer: A meta-analysis. *World J Gastroenterol* 2013; 19(42): 7461-7471 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7461.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7461

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States[1]. Over the past decades, the standard treatment for patients with inoperable locally advanced pancreatic cancer (LAPC) was chemotherapy alone (CT) or chemo-radiation therapy (CRT)[2-4]; however, the median survival time was only 6-9 mo, and less than 10% of patients survived for 2 years[5]. Definitive results of the 2000-01 FFCD/SFRO study, in which patients randomly received CRT (60 Gy + 5-fluorouracil + cisplatin + gemcitabine) or CT, showed that the progression free survival (PFS) and overall survival (OS) were not significantly different between these two groups[6]. On the contrary, the Eastern Cooperative Oncology Group (ECOG) study, which randomly divided the LAPC patients into a gemcitabine chemotherapy group and a gemcitabine + radiotherapy group, showed that the CRT group had significantly superior OS over the CT group; meanwhile, treatment-related toxicity in the CRT group was acceptable. In the present study, we analyzed the potential impact of CRT or CT or radiotherapy alone (RT) on the survival using meta-analysis methodologies in an attempt to obtain more robust evidence.

MATERIALS AND METHODS

Literature collection

The search strategy was: exp pancreas/(pancreas.tw.exp); pancreatic neoplasms/(pancreas adj neoplasms, pancreas adj cancers, pancreas adj carcinomas); 30-38 exp drug therapy/exp chemotherapy adjuvant/chemotherapy; chemoradiotherapy (combin adj chemotherapy, concurrent adj chemoradiotherapy; 49-50 exp radiotherapy/exp radiotherapy adjuvant/radiotherapy.

The literature search was conducted in both English and Chinese publications. The data sources included PubMed (1964-2012), EmBase (1964-2012), CNKI (1979-2012), CQVIP(1979-2012), China Journals Full Text Database (1979-2012), websites (*e.g.*, Web of Science) and proceedings of major annual meetings such as ASCO (1995-2012) and CSCO (1995-2012). Internet searches were carried out and bibliographies of included articles were searched. Finally, the Chinese references in the selected articles were reviewed for other relevant studies. The latest updates of serial clinical studies were used. Date of last search was October 15, 2012.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients were pathologically confirmed to have locally advanced pancreatic malignant tumors (Tumors were judged as nonresectable due to extension to regional lymph nodes and/or vascular structures such as the superior mesenteric artery or the celiac trunk or the existence of a portal or superior mesenteric-portal venous confluent thrombosis), which were naive to surgical treatment or other anti-tumor therapies before enrollment; (2) the study was a prospective randomized controlled trial; (3) the interventions only included radiotherapy and/or chemotherapy; (4) the main endpoint was survival, and the observation lasted at least 6 mo, along with survival records; and (5) except for the treatment methods, the treatment group was parallel with the control group.

Exclusion criteria: (1) Patients with metastatic pancreatic cancer; individuals who suffered from relapse after anti-tumor treatment; patients who had previously received surgical treatment; patients with non-LAPC; (2) non-prospective and non-randomized/non-controlled studies; (3) other interventions were applied in addition to radiotherapy and chemotherapy; (4) only local efficacy was evaluated and no data on survival was available; and (5) low-quality studies that had a Jadad score of less than 2.

Quality assessment

Assessment of the included literature: Two reviewers independently conducted methodological quality assessment and data retrieval. Cross-checking was then performed; any disagreements were resolved by discussion and, if necessary, a third reviewer. The methodological quality of the included studies was assessed using the Jadad scoring system: (1) Was the study described as randomized? Were the patients actually randomized into the treatment group or control group, and both the observers and patients did not know which group would be allocated? (2 = the appropriate randomization method was described; 1 = the author claimed the use of a randomization method); (2) Except for the targeted intervention(s), were the other procedures consistent between these two groups? (3) Was the study described as blinded (2 = both the patients and observers were blinded, and the blinding method was described; 1 = the author claimed that a double-blinding method was applied; 0 = not blinded); and (4) Was there a description of exclusion bias (*i.e.*, systematical difference of the withdrawals and drop-outs between these two groups; scored 0 or 1 for any reason of drop-outs). The final score ranged from 1 to 5. Studies scored 3 or higher were judged as “high-quality”, and those scored 1 or 2 as “poor-quality”. The criteria for methodological quality analysis were developed: (1) randomization; (2) blinding; (3) withdrawal or drop-outs; (4) allocation concealment; and (5) adoption of intentional analysis. The quality of the included literature was presented as weightings in a forest plot.

Assessment of the result quality: Using evidence-based medicine principles, we conducted a quality assessment

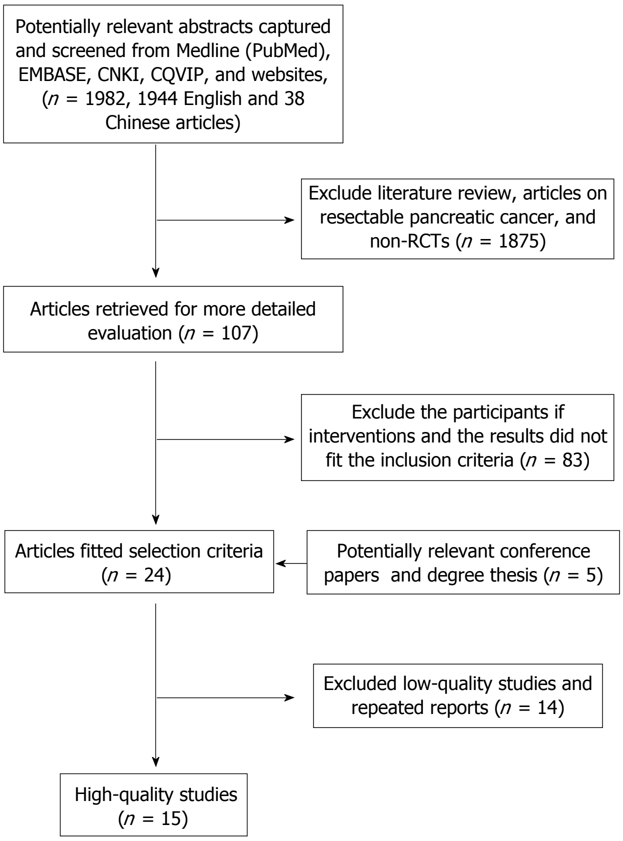

Potentially relevant abstracts captured and screened from Medline (PubMed), EMBASE, CNKI, CQVIP, and websites, (n = 1982, 1944 English and 38 Chinese articles)
Exclude literature review, articles on resectable pancreatic cancer, and non-RCTs (n = 1875)
Articles retrieved for more detailed evaluation (n = 107)
Exclude the participants if interventions and the results did not fit the inclusion criteria (n = 83)
Articles fitted selection criteria (n = 24)
Potentially relevant conference papers and degree thesis (n = 5)
Excluded low-quality studies and repeated reports (n = 14)
High-quality studies (n = 15)

Figure 1 Flow diagram depicting the process of identification and inclusion of selected studies. RCT: Randomized controlled trials.

on the design of evidence sources and finally obtained the quality of the meta-analysis results.

Statistical analysis

Meta-analysis was performed using RevMan4.2 Software. For clinical trials reporting the summary measures or Kaplan-Meier curves, we applied the published statistical methods to analyze the risk ratio and the variance of the temporal data that cause the events[7,8]. The statistical heterogeneity of the clinical trials was analyzed using χ^2 test and I^2 test; $P > 0.05$ during χ^2 test indicates low heterogeneity. The primary analysis was completed using a fixed-effects model. If there was a significant statistical heterogeneity, a secondary confirmatory analysis was performed using a random-effects model. Meanwhile, subgroup analysis was performed to determine whether the results were affected by different interventions. By analyzing the difference between combination therapy and CT/RT, we tried to understand their different impacts on the prognosis.

RESULTS

Search results

The flow chart of this study is shown in Figure 1. Reviews, articles about resectable pancreatic cancer, non-randomized controlled trials (RCTs), low-quality studies and repeated reports were excluded. Finally, both reviewers agreed to include 15 RCTs involving 1128 patients in the meta-analysis.

All of them were published in peer-reviewed international and domestic journals. The cases had been followed up for 6-36 mo. All the studies were prospective, randomized, and controlled trials. One article was claimed to be randomized, with a Jadad score of only 2, and therefore the quality was low. The remaining 14 studies scored 3-4, and belonged to high-quality literature, among which two articles used the blinding method. The meta-analysis results are listed in Table 1. There were no duplicates or mis-citations.

Statistical analysis

Meta-analysis on radiochemotherapy and CT/RT for LAPC: Of these 15 RCTs (n = 1128), 12 (n = 964) reported the 6-mo survival; 14 (n = 1098) reported the 12-mo survival; 9 (n = 805) reported the 18-mo survival; the 6-, 12-, and 18-mo survival was 419, 261, and 119 cases, respectively, in the CRT group, and 238, 127, and 43 cases, respectively, in the CT group/RT group. P value was larger than 0.05 in the meta-analysis on the heterogeneity of the 6-mo survival, and a fixed-effects model was used to merge the data. P values were lower than 0.05 in the meta-analysis on the heterogeneity of the 12- and 18-mo survivals, and a random-effects model was used to merge the data. The CRT group had superior survival over the CT/RT group, while the 18-mo survival showed no such significant difference (OR = 1.78, 1.77, and 1.74; 95%CI: 1.33-2.38, 1.08-2.88, and 0.71-4.27, P = 0.0001, 0.02, and 0.23) (Table 2, Figure 2A).

Subgroup analysis on the efficacy of radiochemotherapy and CT/RT for LAPC: Seven RCTs (n = 371) reported the 6-mo survival of patients after combination treatment or CT, 7 (n = 479) reported the 12-mo survival, and 5 (n = 354) reported the 18-mo survival. The 6-, 12-, and 18-mo survivals were reported in 129, 95, and 32 cases, respectively, in the CRT group, and 119, 91, and 37 cases, respectively, in the CT group. P value was larger than 0.05 in the meta-analysis on the heterogeneity of 6-mo survival, and a fixed-effects model was used to merge the data. P values were lower than 0.05 in the meta-analysis on the heterogeneity of the 12- and 18-mo survivals, and a random-effects model was used to merge the data. The 6-, 12-, and 18-mo survivals were not significantly different between the CRT group and the CT group (OR = 1.52, 1.49, and 1.07; 95%CI: 0.97-2.36, 0.77-2.88, and 0.33-3.45, P = 0.07, 0.23, and 0.91) (Figure 2B).

Five RCTs (n = 476) reported the 6-mo survival after combination treatment or RT, 7 (n = 610) reported the 12-mo survival, 4 (n = 451) reported the 18-mo survival. The 6-, 12-, and 18-mo survivals were 246, 166, and 87 cases, respectively, in the CRT group, and 70, 36, and 37 cases, respectively, in the RT group. Meta-analysis on the heterogeneity of the 6-, 12-, and 18-mo survivals showed that the P values were larger than 0.05, and a

Table 1 Characteristics of the eligible trials included in the meta-analysis

Ref.	Treatment arms	No. of participants	Sex (male/ female)	Median age (yr)	PS 0-2/KPS ⩾ 50	Pathology (W/M/ P/other)	Location of primary tumor(head of pancreas/other)	Jad score
Loehrer *et al*[9]	RT 50.4 Gy + GEM *vs*	37	18/19	67	100%	4/6/5/22	12/25	
	GEM	34	19/15	65.3	100%	6/8/7/13	20/14	3
Klaassen *et al*[10]	RT 40 Gy + 5FU *vs* 5FU	44	31/13		100%	NR	NR	
		47	22/25	NR	100%			3
Moertel *et al*[11]	RT 35-40 Gy + saline *vs*	32	NR	NR	NR	NR	NR	
	RT 35-40 Gy + 5FU	32						4
GITSG *et al*[12]	RT 60 Gy *vs* RT 60Gy +	28	12/16	NR	100%	8/17/3/0	10/18	
	5FU *vs* RT 40 Gy + 5FU	32	17/15		100%	6/20/6/0	9/23	3
		29	16/13		100%	6/18/5/0	6/23	
Cohen *et al*[13]	RT 59.4 Gy *vs* RT 59.4 Gy	49	27/22	62	100%	7/21/11/10	NR	
	+ 5FU + MMC	55	37/18	64	100%	12/19/17/7		3
Chauffert *et al*[6]	RT 60 Gy + 5FU + DDP +	59	31/28	60	100%	NR	46/13	
	GEM *vs* GEM	60	34/26	62	100%		40/20	3
Moertel *et al*[14]	RT 60 Gy *vs* RT 60 Gy +	25		54	88%	5/8/2/10	8/17	3
	5FU *vs* RT 40 Gy + 5FU	86		60	95%	20/39/9/14	68/18	
		83		61	95%	13/30/9/31	64/19	
Sun *et al*[15]	RT 45-50 Gy + GEM *vs*	25	32/22	NR	100%	NR	NR	3
	GEM	29			100%			
Sun *et al*[16]	RT 50-60 Gy + GEM *vs*	26	33/23	NR	100%	NR	NR	3
	GEM + DDP	30			100%			
Wu *et al*[17]	RT 48-56 Gy *vs* RT 48-60	31	50/14	57	98%	NR	NR	3
	Gy + GEM + DDP	33		57	99%			
Wu *et al*[18]	RT 60 Gy *vs* RT 50 Gy +	34	43/27	NR	87%	NR	NR	3
	GEM	36			90%			
Ding *et al*[19]	RT 45-50 Gy + 5FU +	25	32/22	NR	100%	NR	NR	3
	GEM *vs* 5FU + GEM	29			100%			
Childs *et al*[20]	RT 35-40 Gy + saline *vs*	12	11/1	58.8	NR	NR	NR	4
	RT 35-40 Gy + 5FU	13	8/5	56.3				
GITSG *et al*[21]	RT 54 Gy + 5FU + SMF	22	8/14	61	100%	NR	3/18	3
	vs SMF	21	8/13	60	100%		3/19	
Hazel *et al*[22]	RT 46 Gy + 5FU *vs* 5FU +	15	10/5	62	NR	NR	NR	2
	CCNU	15	10/5	62				

W: Well differentiated; M: Moderately differentiated; P: Poorly differentiated; SMF: Streptozocin, mitomycin, and 5-fluorouracil (5FU); NR: No report; GEM: Gemcitabine; RT: radiotherapy alone; MMC: Mitomycin C.

fixed-effects model was used to merge the data. The 6-, 12-, and 18-mo survivals were significantly higher in the CRT group than in the RT group (OR = 2.49, 2.42, and 3.86; 95%CI: 1.62-3.82, 1.57-3.74, 1.66-8.99, all $P < 0.01$), (Figure 2C).

Subgroup analysis on the efficacy of CRT > 50Gy and RT for LAPC: Due to the limited number of patients and RCTs, this subgroup analysis only covered the 12-mo survival rate. Five RCTs (n = 409) reported the 12-mo survival of patients after CRT at a dose larger than 50Gy or RT, including 32 cases in the CRT group and 98 cases in the RT group. P value was larger than 0.05 in the meta-analysis on the heterogeneity of the 12-mo survival, and a fixed-effects model was used to pool the data. The CRT group had superior 12-mo survival over the RT group (OR = 2.32; 95%CI: 1.44-3.73, P =0.0005) (Figure 2D).

Subgroup analysis on the efficacy of CRT < 50 Gy and RT for LAPC: Due to the limited number of patients and RCTs, this subgroup analysis was only made on the 6- and 12-mo survival rates. Four RCTs (n = 254) reported the 6-mo survival and 4 (n = 254) reported the 12-mo survival. The 6- and 12-mo survival was reported in 108 and 68 cases in the CRT group, and 42 and 12 cases in the RT group, respectively. P value was larger than 0.05 in the meta-analysis on the heterogeneity of 6- and 12-mo survival, and a fixed-effects model was used to pool the data. The CRT group had superior 6- and 12-mo survival over the RT group (OR = 2.5 and 4.12; 95%CI: 1.45-4.30 and 2.04-8.35, P = 0.0009 and < 0.0001) (Figure 2E).

Due to the limited number of cases and RCTs included, subgroup analysis was not performed on the efficacy of different doses of CRT and CT for LAPC.

Meta-analysis on grade 3-4 treatment-related toxicity: In total, 12 RCTs (n = 874) reported grade 3-4 treatment-related hematologic toxicities, and 10 (n = 713) reported grade 3-4 treatment-related non-hematologic toxicities. Meta-analysis on the heterogeneity showed that the P value was larger than 0.05, and a fixed-effects model was used to merge the data. The CRT group had significantly more grade 3-4 treatment-related hematologic and non-hematologic toxicities than the CT group or RT group (OR = 3.74 and 1.71; 95%CI: 2.56-5.47 and 1.16-2.53, both $P < 0.01$) (Figure 2F).

Table 2 Overall survival and treatment-related toxicity of the eligible trials included in the meta-analysis

Ref.	Treatment arms	Participants	Overall survival (*n*)			3-4 grade treatment-related toxicity (*n*)	
			6 mo	12 mo	18 mo	Hematological	Non-hematological
Loehrer *et al*[9]	RT 50.4 Gy + GEM *vs* GEM	37	28	12	4	29	
		34	26	17	10	26	
Klaassen *et al*[10]	RT 40 Gy + 5FU *vs* 5FU	44	25	14	11	8	NR
		47	28	12	5	1	
Moertel *et al*[11]	RT 35-40 Gy + saline *vs* RT 35-40 Gy + 5FU	32	15	3	0	12	13
		32	20	6	1	24	24
GITSG *et al*[12]	RT 60 Gy *vs* RT 60 Gy + 5FU *vs* RT 40 Gy + 5FU	28	10	2	0		
		32	25	11	3	NR	NR
		29	19	11	4		
Cohen *et al*[13]	RT 59.4 Gy *vs* RT 59.4 Gy + 5FU + MMC	49	28	10	2	5	13
		55	36	17	5	13	17
Chauffert *et al*[6]	RT 60 Gy + 5FU + DDP + GEM *vs* GEM	59	47	20	11	29	12
		60	49	33	21	12	11
Moertel *et al*[14]	RT 60 Gy *vs* RT 60 Gy + 5FU *vs* RT 40 Gy + 5FU	25	13	6	4	4	0
		86	74	52	34	53	22
		83	63	49	40	39	18
Sun *et al*[15]	RT 45-50 Gy + GEM *vs* GEM	25	18	12	NR	5	5
		29	15	7		5	4
Sun *et al*[16]	RT 50-60 Gy + GEM *vs* GEM + DDP	26	21	16	NR	3	6
		30	18	11		3	10
Wu *et al*[17]	RT 48-56 Gy *vs* RT 48-60 Gy + GEM + DDP	31	NR	9	NR	NR	NR
		33		7			
Wu *et al*[18]	RT 60 Gy *vs* RT 50 Gy + GEM	34	NR	5	NR	0	NR
		36		11		2	
Ding *et al*[19]	RT 45-50 Gy + 5FU + GEM *vs* 5FU + GEM	25	18	12	NR	5	5
		29	15	7		5	4
Childs *et al*[20]	RT 35-40 Gy + saline *vs* RT 35-40 Gy + 5FU	12	4	1	NR	2	5
		13	6	2		8	9
GITSG *et al*[21]	RT 54 Gy + 5FU + SMF *vs* SMF	22	19	9	4	14	3
		21	17	4	0	3	3
Hazel *et al*[22]	RT 46 Gy + 5FU *vs* 5FU + CCNU	15	NR	NR	2	NR	NR
		15			1		

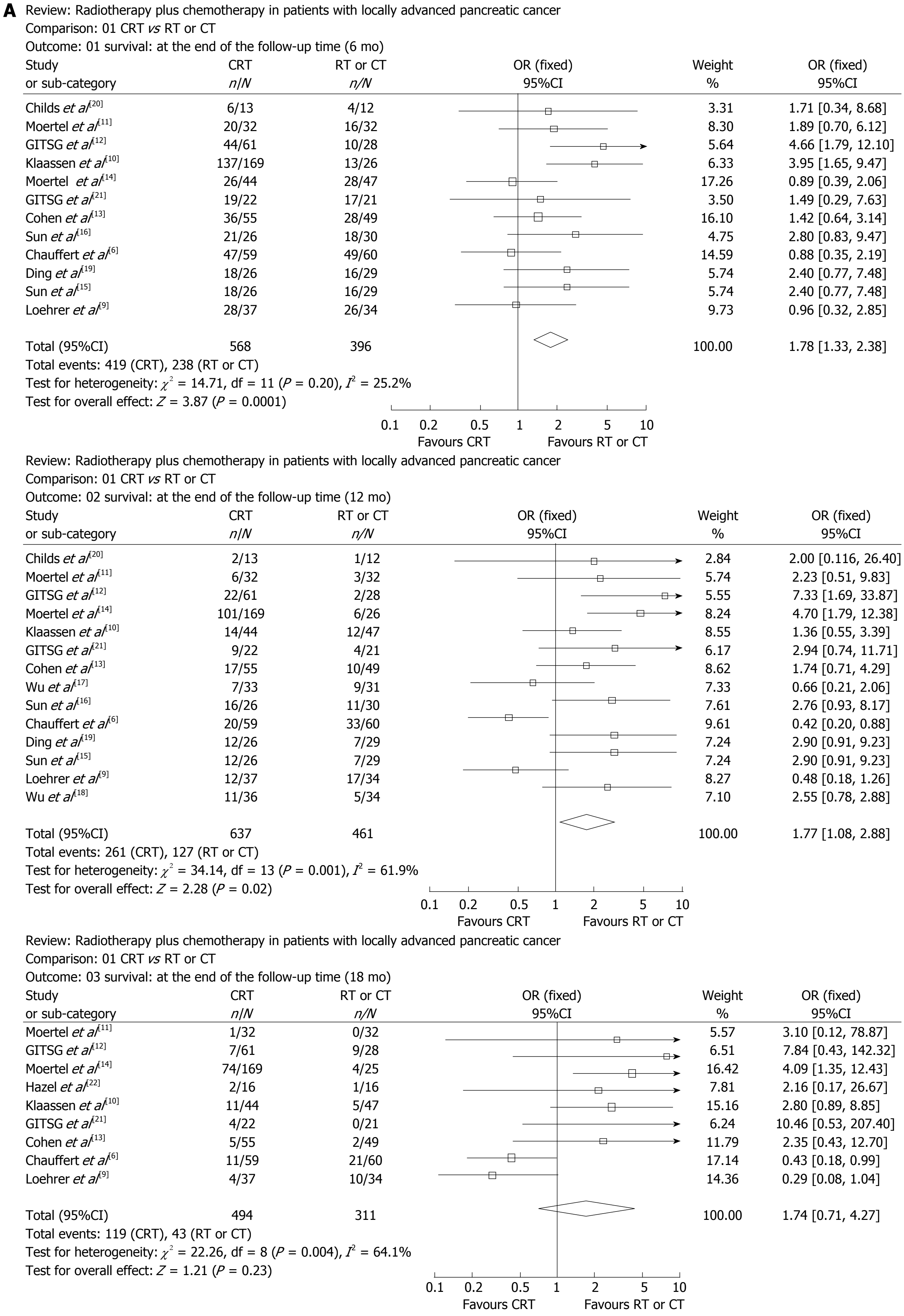
RT: Radiotherapy alone; NR: No report.

Sensitivity analysis

By re-analyzing the results, the sensitivity analysis was designed to re-combine the available studies to explore the effect of a certain factor on the effect, so as to understand the influences of uncertain factors and study design on the aggregate results. To avoid the effect of different chemotherapy or radiotherapy in the different randomized controlled trials on the overall results, subgroup analyses were performed to re-analyze the above studies, and the results were generally consistent with the meta-analysis findings. As shown in the sensitivity analysis, our current meta-analysis neither increased or decreased the efficacy nor exaggerated the efficacy; after the strength of the articles was changed, the results did not become negative or reversed. Therefore, our results were relatively stable and reliable.

DISCUSSION

Pancreatic adenocarcinoma is among the most challenging solid malignancies to treat on account of its propensity for late presentation with inoperable disease, aggressive tumor biology and resistance to chemotherapy[23]. About a third of all pancreatic cancers is found to be locally advanced at the time of diagnosis, LAPC refers to local tumors that have invaded the surrounding normal tissues and can not be surgically resected while no distant metastasis occurs[24]. As shown in early clinical practice, conventional radiotherapy for LAPC often can not improve the efficacy. The availability of three-dimensional conformal radiotherapy and intensity-modulated radiotherapy improves the therapeutic effectiveness, although controversies persist. Using the meta-analysis methodologies, we rigorously screened eligible randomized controlled trials for analysis. As shown in our current meta-analysis: (1) the CRT group had higher 6- and 12-mo survival rates than the RT alone and CT alone group; (2) subgroup analysis showed that the CRT group had higher 6-, 12-, and 18-mo survival rates than CT alone group; (3) subgroup analysis showed that the CRT group had higher 6-, 12-, and 18-mo survival rates than the RT alone group; and (4) CRT group had significantly more grade 3-4 treatment-related hematologic and non-hematologic toxicities than the CT group or RT group. By analyzing the results of 2000 FFCD/SFR0[6], we found that concurrent three-dimensional conformal radiotherapy (total dosage: 60 Gy) with chemotherapy (DDP + 5FU), followed by GMZ chemotherapy achieved a median survival of 8.6 mo and a 1-year survival rate of 32%; the GMZ CT group had a survival of 13 mo and 1-year survival rate of 53%, sug-



Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT *vs* RT or CT
Outcome: 01 survival: at the end of the follow-up time (6 mo)

Study or sub-category	CRT *n*/*N*	RT or CT *n*/*N*	Weight %	OR (fixed) 95%CI
Childs *et al*[20]	6/13	4/12	3.31	1.71 [0.34, 8.68]
Moertel *et al*[11]	20/32	16/32	8.30	1.89 [0.70, 6.12]
GITSG *et al*[12]	44/61	10/28	5.64	4.66 [1.79, 12.10]
Klaassen *et al*[10]	137/169	13/26	6.33	3.95 [1.65, 9.47]
Moertel *et al*[14]	26/44	28/47	17.26	0.89 [0.39, 2.06]
GITSG *et al*[21]	19/22	17/21	3.50	1.49 [0.29, 7.63]
Cohen *et al*[13]	36/55	28/49	16.10	1.42 [0.64, 3.14]
Sun *et al*[16]	21/26	18/30	4.75	2.80 [0.83, 9.47]
Chauffert *et al*[6]	47/59	49/60	14.59	0.88 [0.35, 2.19]
Ding *et al*[19]	18/26	16/29	5.74	2.40 [0.77, 7.48]
Sun *et al*[15]	18/26	16/29	5.74	2.40 [0.77, 7.48]
Loehrer *et al*[9]	28/37	26/34	9.73	0.96 [0.32, 2.85]
Total (95%CI)	568	396	100.00	1.78 [1.33, 2.38]

Total events: 419 (CRT), 238 (RT or CT)
Test for heterogeneity: χ^2 = 14.71, df = 11 (*P* = 0.20), I^2 = 25.2%
Test for overall effect: *Z* = 3.87 (*P* = 0.0001)

Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT *vs* RT or CT
Outcome: 02 survival: at the end of the follow-up time (12 mo)

Study or sub-category	CRT *n*/*N*	RT or CT *n*/*N*	Weight %	OR (fixed) 95%CI
Childs *et al*[20]	2/13	1/12	2.84	2.00 [0.116, 26.40]
Moertel *et al*[11]	6/32	3/32	5.74	2.23 [0.51, 9.83]
GITSG *et al*[12]	22/61	2/28	5.55	7.33 [1.69, 33.87]
Moertel *et al*[14]	101/169	6/26	8.24	4.70 [1.79, 12.38]
Klaassen *et al*[10]	14/44	12/47	8.55	1.36 [0.55, 3.39]
GITSG *et al*[21]	9/22	4/21	6.17	2.94 [0.74, 11.71]
Cohen *et al*[13]	17/55	10/49	8.62	1.74 [0.71, 4.29]
Wu *et al*[17]	7/33	9/31	7.33	0.66 [0.21, 2.06]
Sun *et al*[16]	16/26	11/30	7.61	2.76 [0.93, 8.17]
Chauffert *et al*[6]	20/59	33/60	9.61	0.42 [0.20, 0.88]
Ding *et al*[19]	12/26	7/29	7.24	2.90 [0.91, 9.23]
Sun *et al*[15]	12/26	7/29	7.24	2.90 [0.91, 9.23]
Loehrer *et al*[9]	12/37	17/34	8.27	0.48 [0.18, 1.26]
Wu *et al*[18]	11/36	5/34	7.10	2.55 [0.78, 2.88]
Total (95%CI)	637	461	100.00	1.77 [1.08, 2.88]

Total events: 261 (CRT), 127 (RT or CT)
Test for heterogeneity: χ^2 = 34.14, df = 13 (*P* = 0.001), I^2 = 61.9%
Test for overall effect: *Z* = 2.28 (*P* = 0.02)

Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT *vs* RT or CT
Outcome: 03 survival: at the end of the follow-up time (18 mo)

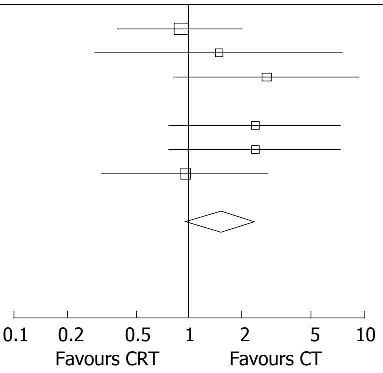
Study or sub-category	CRT *n*/*N*	RT or CT *n*/*N*	Weight %	OR (fixed) 95%CI
Moertel *et al*[11]	1/32	0/32	5.57	3.10 [0.12, 78.87]
GITSG *et al*[12]	7/61	9/28	6.51	7.84 [0.43, 142.32]
Moertel *et al*[14]	74/169	4/25	16.42	4.09 [1.35, 12.43]
Hazel *et al*[22]	2/16	1/16	7.81	2.16 [0.17, 26.67]
Klaassen *et al*[10]	11/44	5/47	15.16	2.80 [0.89, 8.85]
GITSG *et al*[21]	4/22	0/21	6.24	10.46 [0.53, 207.40]
Cohen *et al*[13]	5/55	2/49	11.79	2.35 [0.43, 12.70]
Chauffert *et al*[6]	11/59	21/60	17.14	0.43 [0.18, 0.99]
Loehrer *et al*[9]	4/37	10/34	14.36	0.29 [0.08, 1.04]
Total (95%CI)	494	311	100.00	1.74 [0.71, 4.27]

Total events: 119 (CRT), 43 (RT or CT)
Test for heterogeneity: χ^2 = 22.26, df = 8 (*P* = 0.004), I^2 = 64.1%
Test for overall effect: *Z* = 1.21 (*P* = 0.23)

B Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT *vs* RT or CT
Outcome: 04 survival: at the end of the follow-up time (6 mo) CRT *vs* CT

Study or sub-category	CRT *n*/*N*	RT or CT *n*/*N*	OR (fixed) 95%CI	Weight %	OR (fixed) 95%CI
Klaassen *et al*[10]	25/44	28/47		36.94	0.89 [0.39,2.06]
GITSG *et al*[21]	19/22	17/21		7.50	1.49 [0.29, 7.63]
Sun *et al*[15]	21/26	18/30		10.16	2.80 [0.83, 9.47]
Chauffert *et al*[6]	0/1	0/1		0.00	3.95 [1.65, 9.47]
Ding *et al*[19]	18/26	16/29		12.29	2.40 [0.77, 7.48]
Sun *et al*[15]	18/26	16/29		12.29	2.40 [0.77, 7.48]
Loehrer *et al*[9]	28/37	26/34		20.83	0.96 [0.32, 2.85]
Total (95%CI)	180	191		100.00	1.52 [0.97, 2.36]

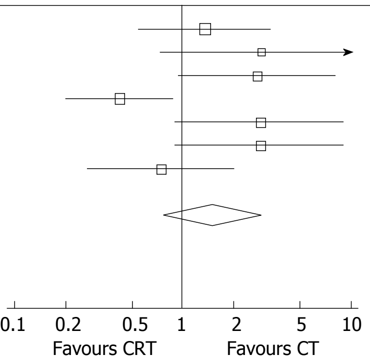
Total events: 129 (CRT), 119 (CT)
Test for heterogeneity: $\chi^2 = 4.46$, df = 5 ($P = 0.49$), $I^2 = 0\%$)
Test for overall effect: $Z = 1.83$ ($P = 0.07$)



Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT *vs* RT or CT
Outcome: 05 survival: at the end of the follow-up time (12 mo) CRT *vs* CT

Study or sub-category	CRT *n*/*N*	RT or CT *n*/*N*	OR (fixed) 95%CI	Weight %	OR (fixed) 95%CI
Klaassen *et al*[10]	14/44	12/47		15.76	1.36 [0.55, 3.39]
GITSG *et al*[21]	9/22	4/22		11.31	2.94 [0.74, 11.71]
Sun *et al*[16]	16/26	11/30		14.00	2.76 [0.93, 8.17]
Chauffert *et al*[6]	20/59	33/60		17.56	0.42 [0.20, 0.88]
Ding *et al*[19]	12/25	7/29		13.30	2.90 [0.91, 9.23]
Sun *et al*[15]	12/25	7/29		13.30	2.90 [0.91, 9.23]
Loehrer *et al*[9]	12/28	17/34		14.78	0.75 [0.27, 2.05]
Total (95%CI)	229	250		100.00	1.49 [0.77, 2.88]

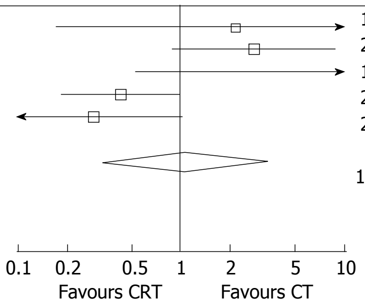
Total events: 95 (CRT), 91 (CT)
Test for heterogeneity: $\chi^2 = 16.96$, df = 6 ($P = 0.009$), $I^2 = 64.6\%$
Test for overall effect: $Z = 1.20$ ($P = 0.23$)



Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT *vs* RT or CT
Outcome: 06 survival: at the end of the follow-up time (18 mo) CRT *vs* CT

Study or sub-category	CRT *n*/*N*	RT or CT *n*/*N*	OR (fixed) 95%CI	Weight %	OR (fixed) 95%CI
Hazel *et al*[22]	2/15	1/15		13.02	2.15 [0.17, 26.67]
Klaassen *et al*[10]	11/44	5/47		24.89	2.80 [0.89, 8.86]
GITSG *et al*[21]	4/22	0/21		10.44	10.46 [0.53, 207.40]
Chauffert *et al*[6]	11/59	21/60		28.03	0.43 [0.18, 0.99]
Loehrer *et al*[9]	4/37	10/34		23.62	0.29 [0.08, 1.04]
Total (95%CI)	177	177		100.00	1.07 [0.33, 3.45]

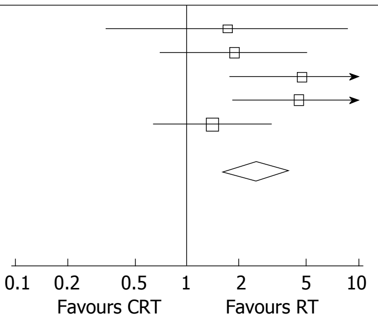
Total events: 32 (CRT), 37 (CT)
Test for heterogeneity: $\chi^2 = 12.65$, df = 4 ($P = 0.01$), $I^2 = 68.4\%$
Test for overall effect: $Z = 0.12$ ($P = 0.91$)

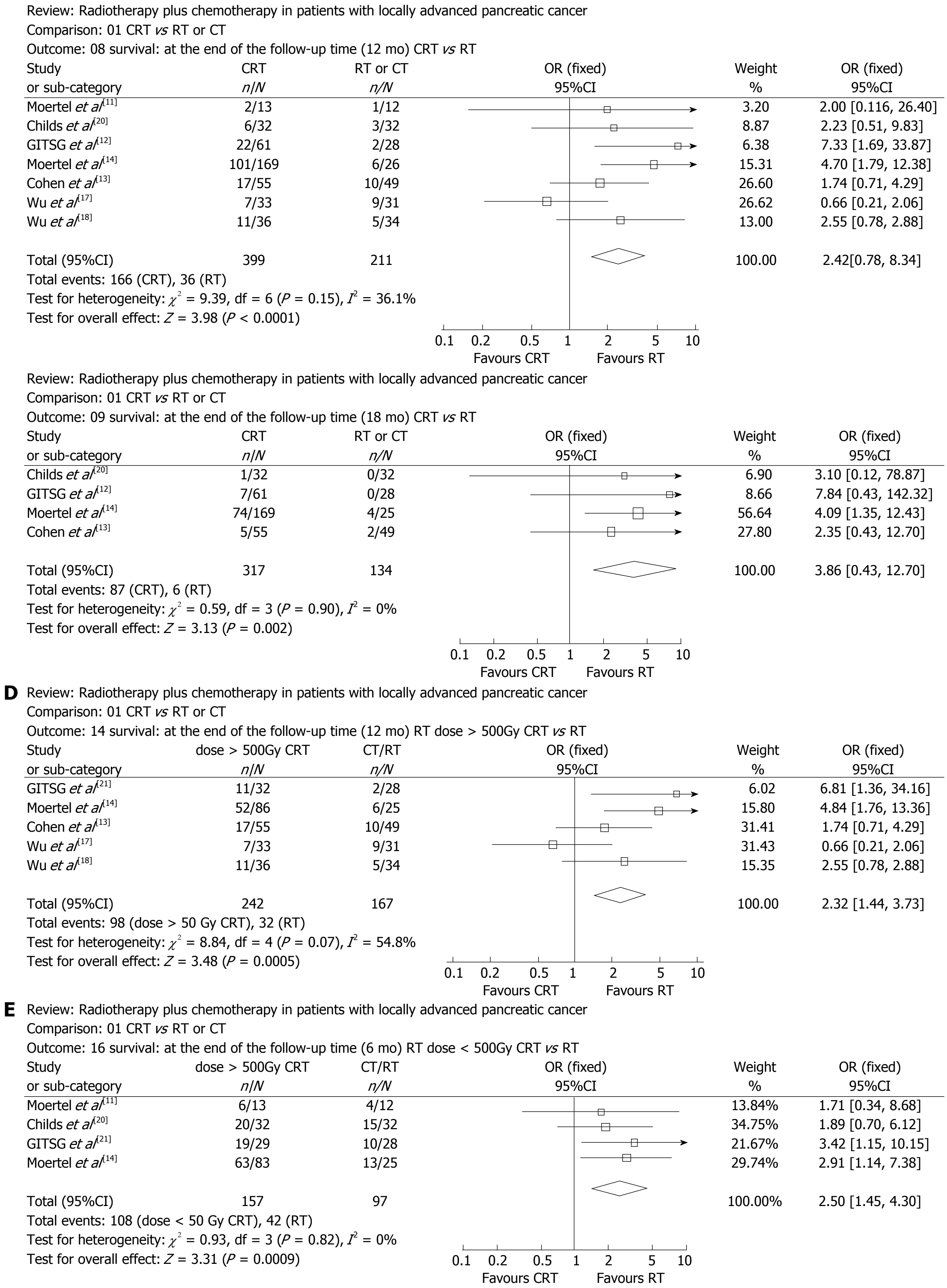


C Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT *vs* RT or CT
Outcome: 07 survival: at the end of the follow-up time (6 mo) CRT *vs* RT

Study or sub-category	CRT *n*/*N*	RT or CT *n*/*N*	OR (fixed) 95%CI	Weight %	OR (fixed) 95%CI
Moertel *et al*[11]	6/13	4/12		8.68	1.71 [0.34, 8.68]
Childs *et al*[20]	20/32	16/32		21.80	1.89 [0.70, 6.12]
GITSG *et al*[12]	44/61	10/28		14.81	4.66 [1.79, 12.10]
Moertel *et al*[14]	140/169	13/25		15.06	0.89 [0.39, 2.06]
Cohen *et al*[13]	36/5	28/49		39.65	1.42 [0.64, 3.14]
Total (95%CI)	330	136		100.00	2.49 [1.62,3.82]

Total events: 246 (CRT), 70 (RT)
Test for heterogeneity: $\chi^2 = 5.76$, df = 4 ($P = 0.22$), $I^2 = 30.5\%$
Test for overall effect: $Z = 4.15$ ($P < 0.0001$)




Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT vs RT or CT
Outcome: 08 survival: at the end of the follow-up time (12 mo) CRT vs RT
Study or sub-category | CRT n/N | RT or CT n/N | OR (fixed) 95%CI | Weight % | OR (fixed) 95%CI
Moertel et al[11] | 2/13 | 1/12 | 3.20 | 2.00 [0.116, 26.40]
Childs et al[20] | 6/32 | 3/32 | 8.87 | 2.23 [0.51, 9.83]
GITSG et al[12] | 22/61 | 2/28 | 6.38 | 7.33 [1.69, 33.87]
Moertel et al[14] | 101/169 | 6/26 | 15.31 | 4.70 [1.79, 12.38]
Cohen et al[13] | 17/55 | 10/49 | 26.60 | 1.74 [0.71, 4.29]
Wu et al[17] | 7/33 | 9/31 | 26.62 | 0.66 [0.21, 2.06]
Wu et al[18] | 11/36 | 5/34 | 13.00 | 2.55 [0.78, 2.88]
Total (95%CI) | 399 | 211 | 100.00 | 2.42[0.78, 8.34]
Total events: 166 (CRT), 36 (RT)
Test for heterogeneity: χ² = 9.39, df = 6 (P = 0.15), I² = 36.1%
Test for overall effect: Z = 3.98 (P < 0.0001)
0.1 0.2 0.5 1 2 5 10
Favours CRT
Favours RT
Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT vs RT or CT
Outcome: 09 survival: at the end of the follow-up time (18 mo) CRT vs RT
Study or sub-category | CRT n/N | RT or CT n/N | OR (fixed) 95%CI | Weight % | OR (fixed) 95%CI
Childs et al[20] | 1/32 | 0/32 | 6.90 | 3.10 [0.12, 78.87]
GITSG et al[12] | 7/61 | 0/28 | 8.66 | 7.84 [0.43, 142.32]
Moertel et al[14] | 74/169 | 4/25 | 56.64 | 4.09 [1.35, 12.43]
Cohen et al[13] | 5/55 | 2/49 | 27.80 | 2.35 [0.43, 12.70]
Total (95%CI) | 317 | 134 | 100.00 | 3.86 [0.43, 12.70]
Total events: 87 (CRT), 6 (RT)
Test for heterogeneity: χ² = 0.59, df = 3 (P = 0.90), I² = 0%
Test for overall effect: Z = 3.13 (P = 0.002)
0.1 0.2 0.5 1 2 5 10
Favours CRT
Favours RT
D
Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT vs RT or CT
Outcome: 14 survival: at the end of the follow-up time (12 mo) RT dose > 500Gy CRT vs RT
Study or sub-category | dose > 500Gy CRT n/N | CT/RT n/N | OR (fixed) 95%CI | Weight % | OR (fixed) 95%CI
GITSG et al[21] | 11/32 | 2/28 | 6.02 | 6.81 [1.36, 34.16]
Moertel et al[14] | 52/86 | 6/25 | 15.80 | 4.84 [1.76, 13.36]
Cohen et al[13] | 17/55 | 10/49 | 31.41 | 1.74 [0.71, 4.29]
Wu et al[17] | 7/33 | 9/31 | 31.43 | 0.66 [0.21, 2.06]
Wu et al[18] | 11/36 | 5/34 | 15.35 | 2.55 [0.78, 2.88]
Total (95%CI) | 242 | 167 | 100.00 | 2.32 [1.44, 3.73]
Total events: 98 (dose > 50 Gy CRT), 32 (RT)
Test for heterogeneity: χ² = 8.84, df = 4 (P = 0.07), I² = 54.8%
Test for overall effect: Z = 3.48 (P = 0.0005)
0.1 0.2 0.5 1 2 5 10
Favours CRT
Favours RT
E
Review: Radiotherapy plus chemotherapy in patients with locally advanced pancreatic cancer
Comparison: 01 CRT vs RT or CT
Outcome: 16 survival: at the end of the follow-up time (6 mo) RT dose < 500Gy CRT vs RT
Study or sub-category | dose > 500Gy CRT n/N | CT/RT n/N | OR (fixed) 95%CI | Weight % | OR (fixed) 95%CI
Moertel et al[11] | 6/13 | 4/12 | 13.84% | 1.71 [0.34, 8.68]
Childs et al[20] | 20/32 | 15/32 | 34.75% | 1.89 [0.70, 6.12]
GITSG et al[21] | 19/29 | 10/28 | 21.67% | 3.42 [1.15, 10.15]
Moertel et al[14] | 63/83 | 13/25 | 29.74% | 2.91 [1.14, 7.38]
Total (95%CI) | 157 | 97 | 100.00% | 2.50 [1.45, 4.30]
Total events: 108 (dose < 50 Gy CRT), 42 (RT)
Test for heterogeneity: χ² = 0.93, df = 3 (P = 0.82), I² = 0%
Test for overall effect: Z = 3.31 (P = 0.0009)
0.1 0.2 0.5 1 2 5 10
Favours CRT
Favours RT

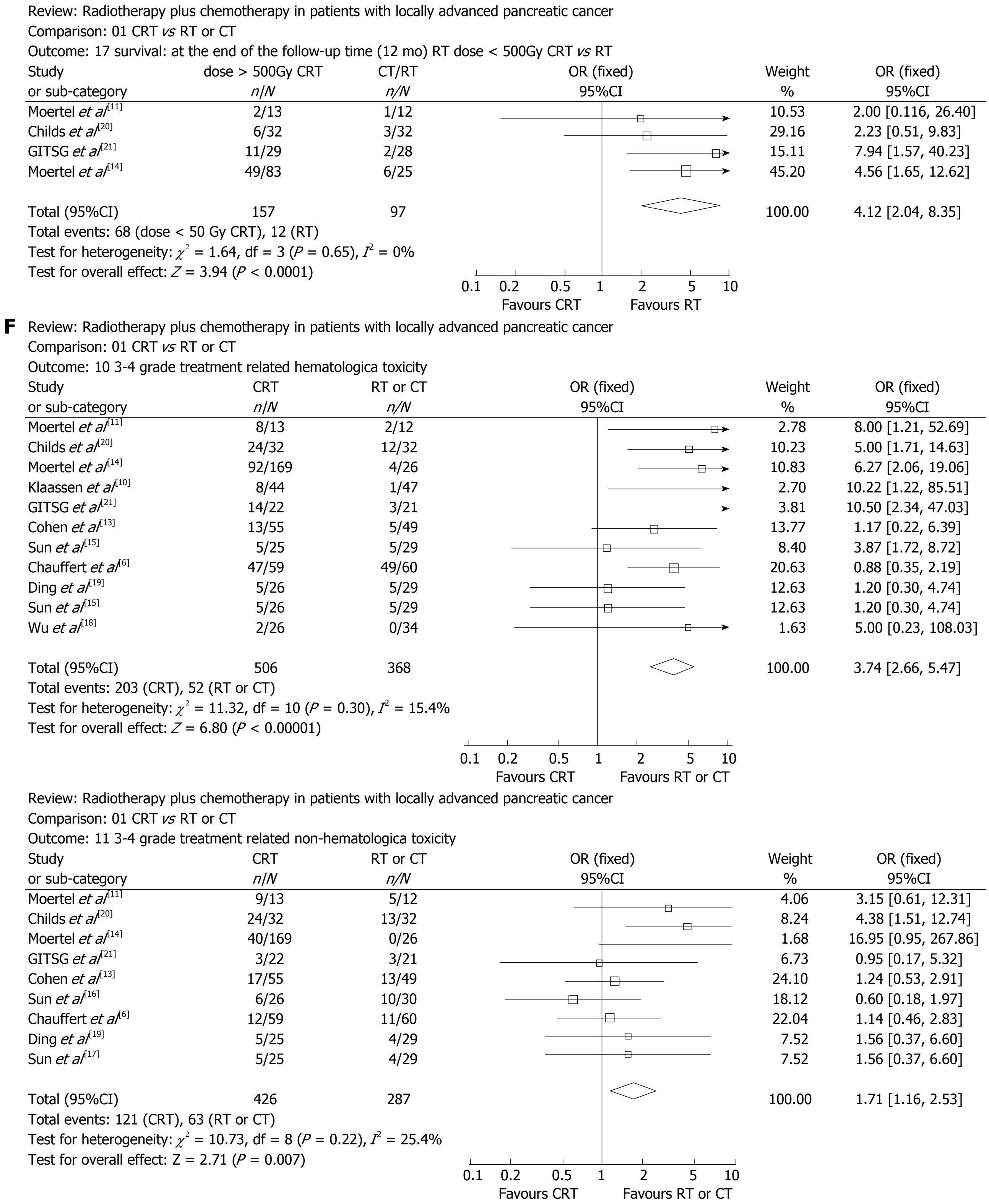


Figure 2 Meta-analysis. A: On chemotherapy plus radiotherapy (CRT) *vs* chemotherapy alone (CT)/radiotherapy alone (RT) for locally advanced pancreatic carcinoma (LAPC) in the 6-, 12-, and 18-mo survivals; B: On CRT *vs* CT for LAPC in the 6-, 12-, and 18-mo survivals; C: On CRT *vs* RT for LAPC in the 6-, 12-, and 18-mo survivals; D: On CRT > 50 Gy *vs* RT for LAPC in the 12-mo survivals; E: On CRT < 50 Gy *vs* RT for LAPC in the 6- and 12-mo survivals; F: On CRT *vs* CT/RT for LAPC in grade 3-4 treatment-related toxicities.

gesting that the combination therapy did not change the efficacy. Meanwhile, the 60 Gy dosage induced more complications and therefore increased the mortality and decreased the survival. The CRT protocol applied in this trial used high radiotherapy and chemotherapy dosages; furthermore, the radiation fields included not only the cancer foci but also the peripancreatic, hilar and celiac trunk lymph nodes that had high metastatic potential (but not confirmed), which not only remarkably increased the radiotherapy-related toxic reactions but also shortened the survival. Similarly, radiochemotherapy was applied in the ECOG4201 trial, although the radiotherapy dose was lowered to 50.4 Gy. Compared with CT, concurrent radio-chemotherapy prolonged the survival (11 mo *vs* 9.2 mo) and yielded a higher 1-year survival rate (50% *vs* 32%)[25]. During concurrent radiochemotherapy, the sequencing of radiotherapy and chemotherapy can also affect the clinical efficacy. In the GERCOR study, 181 patients received 4 cycles of chemotherapy initially, among whom 53 experienced disease progression; the remaining 128 patients without disease progression were divided into a concurrent CRT group (n = 72) and a CT group (n = 56). The results showed that the PFS and OS were improved in the concurrent CRT group (10.8 mo and 15 mo, respectively), and these were significantly longer than those in the CT group (7.4 mo and 11.7 mo, respectively) (P = 0.005, P = 0.0009, respectively), indicating that radiotherapy combined with chemotherapy can remarkably improve the survival[26].

In summary, as shown in this study, the combination of chemotherapy and radiotherapy can prolong the long-term survival although it may also increase the treatment-related toxicity. More reasonably designed randomized controlled trials should be conducted to further elucidate the optimal radiotherapy dosage, the use of gemcitabine or 5-FU, and more specific chemotherapy protocols. Chemotherapy may be started for several cycles; if no disease progression occurs, concurrent radiochemotherapy may be used. By doing so, we may rule out patients with rapid disease progression to avoid the "double whammy" from chemotherapy. Along with the improvement of radiotherapy technology, the optimization of the sequencing of radiochemotherapy, and the definition of target population, the efficacy of radiochemotherapy for pancreatic cancer will be further improved, and multidisciplinary and individualized radiochemotherapy for LAPC will play a more important role.

COMMENTS

Background

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States. Over the past decades, the standard treatment for patients with locally advanced pancreatic carcinoma (LAPC) was chemotherapy alone or chemo-radiation therapy. However, the efficacy of the combined radiotherapy and chemotherapy is currently unclear.

Research frontiers

Over the past decades, many studies have been performed to understand the role of chemotherapy or radiation therapy alone and chemo-radiation therapy in LAPC. Some trials showed no improvement in local progression or progression-free or overall survival with the addition of radiation therapy to chemotherapy in patients with LAPC; however, some trials demonstrated improved overall survival, with acceptable toxicity. Moreover, several systematic reviews were recently published to investigate the role of chemo-radiation therapy in LAPC. However, these reviews were methodologically insufficient and thus could not achieve a comprehensive conclusion.

Innovations and breakthroughs

Based on this meta-analysis, chemo-radiation therapy was superior in the 6- and 12-mo survivals to the radiation therapy group or chemotherapy group alone. Similar results were indicated in the subgroup analyses. The radiation therapy group had significantly more grade 3-4 treatment-related hematologic and non-hematologic toxicities than the radiation therapy group or chemotherapy group alone. These findings were not presented clearly in previous systematic reviews.

Applications

The combination of chemotherapy and radiotherapy can prolong the long-term survival of the patients with LAPC although it may also increase the treatment-related toxicity. Chemotherapy may be started for several cycles; if no disease progression occurs, concurrent radiochemotherapy may be used.

Terminology

Locally advanced pancreatic malignant tumors were judged as nonresectable due to extension to regional lymph nodes and/or vascular structures such as the superior mesenteric artery or the celiac trunk or the existence of a portal or superior mesenteric-portal venous confluent thrombosis. Over the past decades, the standard treatment for patients with inoperable locally advanced pancreatic cancer was chemotherapy alone or chemo-radiation therapy.

Peer review

This is a well written manuscript analyzing therapeutic management of pancreatic cancer. In this manuscript, the authors compared the long-term clinical efficacy of chemotherapy plus radiotherapy with that of radiotherapy alone or chemotherapy alone for locally advanced pancreatic carcinoma. The data were well collected, and analyzed.

REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]

2 **Hu J**, Zhao G, Wang HX, Tang L, Xu YC, Ma Y, Zhang FC. A meta-analysis of gemcitabine containing chemotherapy for locally advanced and metastatic pancreatic adenocarcinoma. *J Hematol Oncol* 2011; **4**: 11 [PMID: 21439076 DOI: 10.1186/1756-8722-4-11]

3 **Yip D**, Karapetis C, Strickland A, Steer CB, Goldstein D. Chemotherapy and radiotherapy for inoperable advanced pancreatic cancer. *Cochrane Database Syst Rev* 2006; (**3**): CD002093 [PMID: 16855985]

4 **Hackert T**, Büchler MW. Pancreatic cancer: advances in treatment, results and limitations. *Dig Dis* 2013; **31**: 51-56 [PMID: 23797123 DOI: 10.1159/000347178]

5 **Niederhuber JE**, Brennan MF, Menck HR. The National Cancer Data Base report on pancreatic cancer. *Cancer* 1995; **76**: 1671-1677 [PMID: 8635074]

6 **Chauffert B**, Mornex F, Bonnetain F, Rougier P, Mariette C, Bouché O, Bosset JF, Aparicio T, Mineur L, Azzedine A, Hammel P, Butel J, Stremsdoerfer N, Maingon P, Bedenne L. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. *Ann Oncol* 2008; **19**: 1592-1599 [PMID: 18467316 DOI: 10.1093/annonc/mdn281]

7 **Tierney JF**, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007; **8**: 16 [PMID: 17555582]

8 **Walter SD**. Choice of effect measure for epidemiological data. *J Clin Epidemiol* 2000; **53**: 931-939 [PMID: 11004419]

9 **Loehrer PJ**, Feng Y, Cardenes H, Wagner L, Brell JM, Cella D, Flynn P, Ramanathan RK, Crane CH, Alberts SR, Benson AB. Gemcitabine alone versus gemcitabine plus radiotherapy in patients with locally advanced pancreatic cancer: an Eastern Cooperative Oncology Group trial. *J Clin Oncol* 2011; **29**: 4105-4112 [PMID: 21969502 DOI: 10.1200/JCO.2011.34.8904]

10 **Klaassen DJ**, MacIntyre JM, Catton GE, Engstrom PF, Moertel CG. Treatment of locally unresectable cancer of the stomach and pancreas: a randomized comparison of 5-fluorouracil alone with radiation plus concurrent and maintenance 5-fluorouracil--an Eastern Cooperative Oncology Group study. *J Clin Oncol* 1985; **3**: 373-378 [PMID: 3973648]

11 **Moertel CG**, Childs DS, Reitemeier RJ, Colby MY, Holbrook MA. Combined 5-fluorouracil and supervoltage radiation therapy of locally unresectable gastrointestinal cancer. *Lancet* 1969; **2**: 865-867 [PMID: 4186452]

12 **The Gastrointestinal Tumor Study Group**. A multi-institutional comparative trial of radiation therapy alone and in combination with 5-fluorouracil for locally unresectable pancreatic carcinoma. *Ann Surg* 1979; **189**: 205-208 [PMID: 426553]

13 **Cohen SJ**, Dobelbower R, Lipsitz S, Catalano PJ, Sischy B, Smith TJ, Haller DG. A randomized phase III study of radiotherapy alone or with 5-fluorouracil and mitomycin-C in patients with locally advanced adenocarcinoma of the pancreas: Eastern Cooperative Oncology Group study E8282. *Int J Radiat Oncol Biol Phys* 2005; **62**: 1345-1350 [PMID: 16029791 DOI: 10.1016/j.ijrobp]

14 **Moertel CG**, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, Schutt AJ, Weiland LH, Childs DS, Holbrook MA, Lavin PT, Livstone E, Spiro H, Knowlton A, Kalser M, Barkin J, Lessner H, Mann-Kaplan R, Ramming K, Douglas HO, Thomas P, Nave H, Bateman J, Lokich J, Brooks J, Chaffey J, Corson JM, Zamcheck N, Novak JW. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil: The Gastrointestinal Tumor Study Group. *Cancer* 1981; **48**: 1705-1710 [PMID: 7284971]

15 **Sun YW**, Zhang RH, Zhou JY, Liang J. Effect of gemcitabine plus three dimensionalconformal radiotherapy on locally advanced pancreatic cancer. *Zhonghua Shiyong Yixue Zazhi* 2010; **37**: 11-15

16 **Sun YW**, Zhou JY, An YH, Liang J. The Clinical efficiency of gemcitabine plus three dimensional conformal radiotherapy on locally advanced pancreatic cancer-comparison with gencitabine plus cisplatin. *Xiandai Zhongliu Yixue* 2007; **15**: 826-828 [DOI: 10.3969/j.issn.1672-4992.2007.06.033]

17 **Wu DH**, Chen YQ, Chen LH. Observation of the Effects of 3-dimensional conformal radiotherapy therapy combined with chemotherapy in treatment of pancreatic cancer. *Zhongguo Linchuang Zhongliu Zazhi* 2004; **31**: 449-551 [DOI: 10.3969/j.issn.1000-8179.2004.08.010]

18 **Wu F**, Meng CY. Radiotherapy alone or plus chemotherapy in patients with locally advanced pancreatic cancer. *Zhongguo Shiyong Yiyao Zazhi* 2011; **25**: 117-119

19 **Ding ZJ**, Chen Y, Zhou JY, An YH. The effect of combination chemotherapy of gemcitabine and 5-FU plus three dimensional conformal radiotherapy on locally advanced pancreatic cancer. *Zhongguo Linchuang Shiyong Yixue* 2008; **2**: 42-43

20 **Childs DS**, Moertel CG, Holbrook MA, Reitemeier RJ, Colby MY. Treatment of malignant neoplasms of the gastrointestinal tract with a combination of 5-fluorouracil and radiation: a randomized double-blind study. *Radiology* 1965; **84**: 843-848 [PMID: 14282693]

21 **Gastrointestinal Tumor Study Group**. Treatment of locally unresectable carcinoma of the pancreas: comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. *J Natl Cancer Inst* 1988; **80**: 751-755 [PMID: 2898536]

22 **Hazel JJ**, Thirlwell MP, Huggins M, Maksymiuk A, MacFarlane JK. Multi-drug chemotherapy with and without radiation for carcinoma of the stomach and pancreas: a prospective randomized trial. *J Can Assoc Radiol* 1981; **32**: 164-165 [PMID: 7028759]

23 **Javle M**, Hsueh CT. Recent advances in gastrointestinal oncology--updates and insights from the 2009 annual meeting of the American society of clinical oncology. *J Hematol Oncol* 2010; **3**: 11 [PMID: 20331897 DOI: 10.1186/1756-8722-3-11]

24 **Savir G**, Huber KE, Saif MW. Locally advanced pancreatic cancer. Looking beyond traditional chemotherapy and radiation. *JOP* 2013; **14**: 337-339 [PMID: 23846922 DOI: 10.6092/1590-8577/1677]

25 **Saif MW**. New developments in the treatment of pancreatic cancer. Highlights from the "44th ASCO Annual Meeting". Chicago, IL, USA. May 30 - June 3, 2008. *JOP* 2008; **9**: 391-397 [PMID: 18648128]

26 **Huguet F**, André T, Hammel P, Artru P, Balosso J, Selle F, Deniaud-Alexandre E, Ruszniewski P, Touboul E, Labianca R, de Gramont A, Louvet C. Impact of chemoradiotherapy after disease control with chemotherapy in locally advanced pancreatic adenocarcinoma in GERCOR phase II and III studies. *J Clin Oncol* 2007; **25**: 326-331 [PMID: 17235048]

P- Reviewers: Fietkau R, Lalic H **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7472

World J Gastroenterol 2013 November 14; 19(42): 7472-7475
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

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CASE REPORT

Massive upper gastrointestinal hemorrhage due to invasive hepatocellular carcinoma and hepato-gastric fistula

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Received: June 26, 2013 **Revised:** September 3, 2013
Accepted: September 15, 2013
Published online: November 14, 2013

Abstract

A 36-year-old male Asian immigrant with a history of hepatitis B and hepatitis C related unresectable hepatocellular carcinoma in the left lobe of the liver presented with hematemesis and severe anemia. He was diagnosed with a liver mass that was resected 8 years ago described as a benign tumor in his home country. He had received trans-arterial chemoembolization (TACE) four months ago after subsequent diagnosis of unresectable hepatoma, and currently was receiving chemotherapy with Sorafenib. After resuscitation, a contrast enhanced computerized tomography was performed which showed fistulization of hepatocellular carcinoma into adjacent stomach. This finding was confirmed during endoscopy with direct visualization of the fistulous opening. Hepatocellular carcinoma (HCC) invading the gastrointestinal (GI) tract is rare. We present a case and literature review of HCC with local invasion of the stomach causing massive upper GI bleeding after receiving TACE.

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Key words: Hepatocellular carcinoma; Hepatogastric fistula; Fistula; Upper gastrointestinal bleeding

Core tip: Gastrointestinal bleeding is a common complication of hepatocellular carcinoma (HCC). However, HCC leading to Hepatogastric fistula presenting as massive upper gastrointestinal bleeding is uncommon. Here we report a case of HCC with direct invasion of the stomach leading to massive gastrointestinal bleeding. Patient was managed with selective arterial angiogram and coil embolization to control bleeding. HCC with local metastasis to adjacent structures such gastrointestinal tract carries poor prognosis. With increasing incidence of HCC and recent improvements in the treatment of advanced HCC, this condition may become more common and awareness among clinicians should help consider this condition in the differential diagnosis and prompt management.

Sayana H, Yousef O, Clarkston WK. Massive upper gastrointestinal hemorrhage due to invasive hepatocellular carcinoma and hepato-gastric fistula. *World J Gastroenterol* 2013; 19(42): 7472-7475 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7472.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7472

INTRODUCTION

Hepatocellular carcinoma (HCC) is an aggressive primary malignancy of the liver with 5-year survival rates as low as 5%[1]. It is the third leading causes of cancer deaths across the world and ninth leading cause of cancer deaths in United States[2]. While the incidence of other common cancers is decreasing in the United States, HCC is on the rise with an average annual percentage change of incidence rate of 3.5%[2]. The majority of cases of HCC are caused by chronic liver disease from hepatitis B and C (78%)[2]. Extra-hepatic metastasis is found at the time of diagnosis in up to 15% of patients with HCC[3]. Lung is the most common site of metastasis followed by intra-abdominal lymph nodes, and bone and adrenal glands[3]. Median survival of patients with extra-hepatic manifestations is less than 6 mo[4]. Metastasis to the gastrointestinal (GI) tract is rare with rates of 0.4%-2% of cases[5] and in up to 12% of cases in autopsy series[6]. Here we report a case of HCC with direct invasion of the stomach leading to massive GI bleeding.

CASE REPORT

A 36-year-old Asian male with hepatitis B and hepatitis C associated HCC presented with chronic melena and massive acute upper GI bleeding with large volume hematemesis. He had undergone trans-arterial chemoembolization (TACE) four months ago for a large, diffuse, unresectable, biopsy proven HCC in the left lobe of the liver. Due to side effects from the procedure, patient declined to undergo further TACE. He had been on Sorafenib for the preceding two and one-half months prior to this presentation. Apparently, the patient had a liver mass that was resected in his home country eight years ago, described verbally to him as "a benign" tumor. Vital signs on presentation included BP 131/75 mmHg, pulse 123, RR 32, temperature 96.0 F, and oxygen saturation was 88% on 4 L *via* nasal cannula. On physical exam, he was tachycardic, chest was clear to auscultation; mucus membranes were pale, abdominal exam showed tender hepatomegaly (10 cm below right coastal margin in the mid-clavicular line) and mid-epigastric tenderness. Alpha fetoprotein level was 8.3 ng/mL. Laboratory data revealed hemoglobin of 2.5 g/dL (hematocrit, 10%; mean corpuscular volume 94), leukocytosis (with white blood cell count 16.7), and platelet count of 539. Coagulation profile showed a mildly prolonged INR of 1.6 and activated partial thromboplastin time of 21. Hemoglobin from prior hospitalization 4 mo ago was 13.3 g/dL. The patient was transfused with packed red cells (7 units) and intravenous fluids were administered. Tumor marker studies including α-fetoprotein (7.6 ng/mL), CEA (3.3 ng/mL), and CA 19.9 (< 0.1 U/mL), all of which were normal. Hepatitis B quantitative polymerase chain reaction was 41048 U/mL, hepatitis Be antigen was negative and hepatitis Be antibody was positive. Hepatitis C quantitative PCR was 65 IU/mL.

Abdominal computed tomography with intravenous and oral contrast noted a large left hepatic lobe mass which was increased in size (19 cm × 13 cm) compared to prior mass (16 cm × 10 cm) noted two months earlier. An EGD was performed and revealed a 1.4 cm fistula in the posterior wall of the distal gastric body with stigmata of recent bleeding *via* the fistula (Figure 1). A large amount of air was seen centrally within the mass, which appeared to communicate with the gastric lumen through a 7 mm defect along the lesser curvature near the gastric fundus (Figure 2). Subsequently, a visceral (celiac, superior mesenteric artery, and left gastric artery) arteriogram demonstrated no evidence of active bleeding. However, selective left gastric artery angiogram showed hypervascular blush within the tumor and it was selectively embolized to achieve decreased flow, utilizing a total of three, 6 mm × 10 cm long Azur detachable coils. His hemoglobin stabilized and he was discharged with plans to start second line palliative chemotherapy. He subsequently presented a month later with recurrent GI bleeding for which he had to undergo further selective embolization of celiac artery branches, which showed signs of neovascularization. The patient opted for hospice care 10 mo after diagnosis of HCC and 6 mo after treatment of acute GI bleed from hepatogastric fistula.

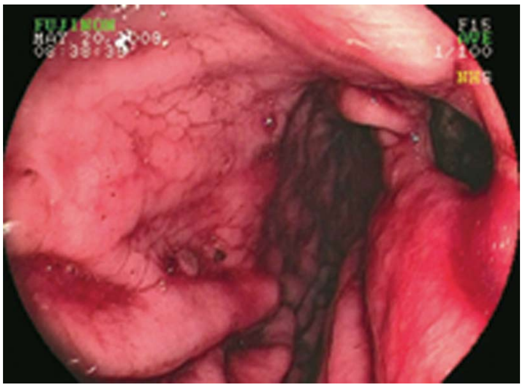

Figure 1 Esophagogastroduodenoscopy image showing the fistulous opening of the hepatocellular carcinoma on the lesser curvature of the stomach.

DISCUSSION

GI bleeding is a common complication in patients with HCC. Common etiologies include peptic ulcer disease, variceal bleeding due to portal hypertension from underlying cirrhosis and/or tumor invasion of portal vein causing thrombosis, and portal hypertensive gastropathy. Yeo *et al*[5] prospectively looked at 55 HCC patients who presented with GI bleeding over a period of 11 mo and found that majority had a non-variceal source of bleeding (53%) and the remainder variceal bleeding. Among patients with a non-variceal source, three patients (0.05%) bled secondary to direct invasion of tumor to gut. GI bleeding secondary to direct invasion by HCC is rare (0.05%-2%)[5,7-9]. Metastatic spread can be due to hematogenous, lymphatic or by direct invasion of the tumor into adjacent organs. The most common site of direct tumor invasion of the GI tract is stomach followed by duodenum and colon. Predisposing factors for GI tract invasion include large liver lesions (> 5 cm in size), subcapsular location and exophytic growth pattern. Suspected predisposing features include prior TACE and/or radiation therapy which can lead to ischemia and inflammatory reactions in the tumor and adjoining bowel wall. These changes can potentially lead to adhesion and local tumor invasion with subsequent fistulization[10,11]. We hypothesize a similar process in our case. Park *et al*[6] found GI tract involvement at the time of diagnosis of HCC

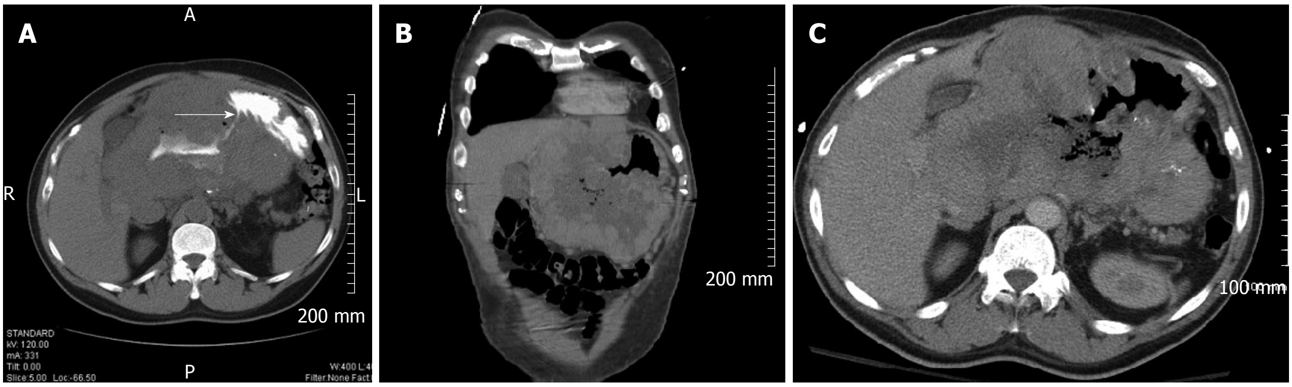

Figure 2 Computerized tomography images of the abdomen. A: Showing large mass in the left lobe of the liver and oral contrast traced into liver (arrow) through fistulous opening in stomach; B: Showing large mass in the left lobe of the liver and fistulous communication between the liver mass and the stomach; C: Showing large mass in the left lobe of the liver and fistulous communication between the liver mass and the stomach. A: Anterior R: Right; L: Left; P: Posterior.

in 7/12 patients without any prior treatment. In our patient, we suspect an exophytic tumor growth pattern causing direct GI tract invasion and pressure necrosis on adjacent organs. This process was likely worsened by TACE/radiation related inflammatory reactions contributing to erosion and fistulization, with subsequent bleeding into the GI tract. Sources of gastrointestinal bleeding noted in the literature have included rupture of hepatoma into the GI tract, bleeding from the intrahepatic portion of the tumor, or bleeding from an involved vessel in the wall of the GI tract. In our case, we endoscopically documented oozing from the hypervascular edges of the gastro-hepatic fistula within the stomach.

The prognosis of patients with HCC and GI involvement is poor because of massive bleeding and/or hepatic failure. In one series, the median survival was 4 wk[7] and in a second study none survived beyond 9 mo[8]. Our patient survived 6 mo. Non-surgical techniques to achieve hemostasis include trans-arterial embolization, endoscopic injection of ethanol or adrenalin, and radiotherapy ablation. Korkolis *et al*[12] reported a case of HCC invading the stomach and upper surface of the pancreas who underwent *en bloc* resection of left lobe of liver with liver mass (15 cm × 12 cm × 9.5 cm), total gastrectomy, distal pancreatectomy, splenectomy, cholecystectomy, radical excision of adjacent lymph node and survival of 16 mo with no local recurrence. Curative surgery is a potential option especially in good surgical candidates with reasonable functional reserve. Surgical intervention has showed prolonged survival with median survival rates of 9.7 mo as opposed to 3.0 mo in non-surgical therapy as reported in other case series[13,14].

In conclusion, due to improvements in chemotherapy and advanced interventional radiologic chemoembolization techniques with improved short term survival in patients with unresectable HCC[15,16], GI tract fistulization and associated upper GI bleeding are likely to become more frequent in the future. This complication may be managed *via* palliative surgical resection, or by angiographic embolization, as was performed in our patient.

REFERENCES

1 **El-Serag HB**, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; **340**: 745-750 [PMID: 10072408 DOI: 10.1056/NEJM199903113401001]

2 **Centers for Disease Control and Prevention (CDC)**. Hepatocellular carcinoma - United States, 2001-2006. *MMWR Morb Mortal Wkly Rep* 2010; **59**: 517-520 [PMID: 20448528]

3 **Uka K**, Aikata H, Takaki S, Shirakawa H, Jeong SC, Yamashina K, Hiramatsu A, Kodama H, Takahashi S, Chayama K. Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma. *World J Gastroenterol* 2007; **13**: 414-420 [PMID: 17230611]

4 **Yoo DJ**, Kim KM, Jin YJ, Shim JH, Ko GY, Yoon HK, Sung KB, Lee JL, Kang YK, Lim YS, Lee HC, Chung YH, Lee YS, Suh DJ. Clinical outcome of 251 patients with extrahepatic metastasis at initial diagnosis of hepatocellular carcinoma: does transarterial chemoembolization improve survival in these patients? *J Gastroenterol Hepatol* 2011; **26**: 145-154 [PMID: 21175808 DOI: 10.1111/j.1440-1746.2010.06341.x]

5 **Yeo W**, Sung JY, Ward SC, Chung SC, Lee WY, Li AK, Johnson PJ. A prospective study of upper gastrointestinal hemorrhage in patients with hepatocellular carcinoma. *Dig Dis Sci* 1995; **40**: 2516-2521 [PMID: 8536505 DOI: 10.1007/BF02220435]

6 **Park MS**, Kim KW, Yu JS, Kim MJ, Yoon SW, Chung KW, Lee JT, Yoo HS. Radiologic findings of gastrointestinal tract involvement in hepatocellular carcinoma. *J Comput Assist Tomogr* 2002; **26**: 95-101 [PMID: 11801910 DOI: 10.1097/00004728-200201000-00014]

7 **Maruyama A**, Murabayashi K, Hayashi M, Nakano H, Isaji S, Uehara S, Kusuda T, Miyahara S, Kondo A, Yabana T. Hepatocellular carcinoma complicated by gastrointestinal hemorrhage caused by direct tumor invasion of stomach. *J Hepatobiliary Pancreat Surg* 1999; **6**: 90-93 [PMID: 10436243 DOI: 10.1007/s005340050089]

8 **Nicoll AJ**, Ireton HJ, Crotty B. Gastrointestinal bleeding from hepatocellular carcinoma invading the stomach. *J Gastroenterol Hepatol* 1994; **9**: 533-535 [PMID: 7827310 DOI: 10.1111/j.1440-1746.1994.tb01290.x]

9 **Lynch P**, Green L, Jordan PH, Graham DY. Hepatocellular carcinoma metastatic to the stomach presenting as bleeding multiple craterogenic ulcers. *Am J Gastroenterol* 1989; **84**: 653-655 [PMID: 2543213]

10 **Chen LT**, Chen CY, Jan CM, Wang WM, Lan TS, Hsieh MY, Liu GC. Gastrointestinal tract involvement in hepatocellular carcinoma: clinical, radiological and endoscopic studies. *Endoscopy* 1990; **22**: 118-123 [PMID: 2162757 DOI: 10.1055/s-2007-1012815]

11 **Lin CP**, Cheng JS, Lai KH, Lo GH, Hsu PI, Chan HH, Hsu JH, Wang YY, Pan HB, Tseng HH. Gastrointestinal metastasis in hepatocellular carcinoma: radiological and endoscopic studies of 11 cases. *J Gastroenterol Hepatol* 2000; **15**: 536-541 [PMID: 10847441 DOI: 10.1046/j.1440-1746.2000.02152.x]

12 **Korkolis DP**, Aggeli C, Plataniotis GD, Gontikakis E, Zerbinis H, Papantoniou N, Xinopoulos D, Apostolikas N, Vassilopoulos PP. Successful en bloc resection of primary hepatocellular carcinoma directly invading the stomach and pancreas. *World J Gastroenterol* 2009; **15**: 1134-1137 [PMID: 19266609 DOI: 10.3748/wjg.15.1134]

13 **Fujii K**, Nagino M, Kamiya J, Uesaka K, Sano T, Yuasa N, Oda K, Nimura Y. Complete resection of hepatocellular carcinoma with direct invasion to the stomach remnant. *J Hepatobiliary Pancreat Surg* 2004; **11**: 441-444 [PMID: 15619024 DOI: 10.1007/s00534-004-0926-3]

14 **Chen CY**, Lu CL, Pan CC, Chiang JH, Chang FY, Lee SD. Lower gastrointestinal bleeding from a hepatocellular carcinoma invading the colon. *J Clin Gastroenterol* 1997; **25**: 373-375 [PMID: 9412926 DOI: 10.1097/00004836-199707000-00019]

15 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

16 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]

P- Reviewer: Helling T **S- Editor**: Qi Y
L- Editor: A **E- Editor:** Liu XM

WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7476

World J Gastroenterol 2013 November 14; 19(42): 7476-7479
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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CASE REPORT

Pseudomembranous colitis associated with a triple therapy for *Helicobacter pylori* eradication

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Author contributions: Trifan A and Stanciu C co-wrote the manuscript; Cojocariu C, Singeap AM, Dorobat C and Grigore L managed the patients; Girleanu I and Sfarti C contributed to the acquisition of data; all authors read and approved the final version of the manuscript.

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Received: June 5, 2013 **Revised:** August 18, 2013
Accepted: September 15, 2013
Published online: November 14, 2013

Abstract

Helicobacter pylori (*H. pylori*) is one of the most common chronic bacterial infections in humans, affecting half of world's population. Therapy for *H. pylori* infection has proven to be both effective and safe. The one-week triple therapy including proton pump inhibitor, clarithromycin, and amoxicillin or metronidazole is still recommended as a first-line treatment to eradicate *H. pylori* infection in countries with low clarithromycin resistance. Generally, this therapy is well-tolerated, with only a few and usually minor side effects. However, rare but severe adverse effects such as pseudomembranous colitis have been reported, *Clostridium difficile* (*C. difficile*) infection being the main causative factor in all cases. We report the cases of two women who developed pseudomembranous colitis after a 1-wk triple therapy consisting of pantoprazole 20 mg *bid*, clarithromycin 500 mg *bid*, and amoxicillin 1 g *bid* to eradicate *H. pylori* infection. A limited colonoscopy showed typical appearance of pseudomembranous colitis, and the stool test for *C. difficile* toxins was positive. Rapid resolution of symptoms and negative *C. difficile* toxins were obtained in both patients with oral vancomycin. No relapse occurred during a four and eleven-month, respectively, follow up. These cases suggest that physicians should have a high index of suspicion for pseudomembranous colitis when evaluate patients with diarrhea following *H. pylori* eradication therapy.

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Key words: *Helicobacter pylori* eradication; Triple therapy; *Clostridium difficile*; Pseudomembranous colitis; Vancomycin

Core tip: Herein are described the cases of two elderly women who developed pseudomembranous colitis after one-week triple therapy consisting of pantoprazole (20 mg *bid*), clarithromycin (500 mg *bid*), and amoxicillin (1 g *bid*) to eradicate *Helicobacter pylori* (*H. pylori*) infection. After a 10-d treatment with oral vancomycin (125 mg every 6 h) both patients had complete resolution of symptoms and negative stool test for *Clostridium difficile* toxins. Clinicians should have a high index of suspicion of pseudomembranous colitis as a rare, but severe complication of *H. pylori* therapy.

Trifan A, Girleanu I, Cojocariu C, Sfarti C, Singeap AM, Dorobat C, Grigore L, Stanciu C. Pseudomembranous colitis associated with a triple therapy for *Helicobacter pylori* eradication. *World J Gastroenterol* 2013; 19(42): 7476-7479 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7476.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7476

INTRODUCTION

Helicobacter pylori (*H. pylori*) is one of the most common chronic bacterial infections in humans, affecting half of the world's population. Its prevalence is high in developing countries and low in the developed ones[1]. *H. pylori* eradication therapy is supported by numerous consensus groups around the world, and the treatment of millions of infected subjects has demonstrated that such strategy is both effective and safe. The spectrum of indications for *H. pylori* eradication therapy has steadily extended over the last decade[2] with a resultant increase in its use. The one-week triple therapy including proton pump inhibitor (PPI), clarithromycin and amoxicillin or metronidazole proposed at the first Maastricht conference[3] to eradicate *H. pylori* is still recommended as the first-line treatment by the recent Maastricht Ⅳ consensus conference[2] in countries with clarithromycin resistance rate under 15%-20% (*e.g.*, Northern European countries)[4]. Eradication rates with standard triple therapy have fallen to 70%-80% over the past few years, mainly due to increasing resistance to clarithromycin[5]. Generally, this therapy is well-tolerated, with only a few and usually minor side effects (*e.g.*, nausea, metallic taste). However, severe adverse effects such as pseudomembranous colitis have been reported[6-13], *Clostridium difficile* (*C. difficile*) being the main causative agent in all cases.

We report the cases of two elderly women who developed pseudomembranous colitis after one-week triple therapy with pantoprazole, clarithromycin, and amoxicillin for *H. pylori* infection.

CASE REPORT

The first case is a 70-year-old woman who was referred to our department with a 10-d history of watery diarrhea (6-12 stools per day) and crampy abdominal pain. Her medical history included hypertension, chronic gastritis *H. pylori* positive, and colonic diverticulosis (previously diagnosed on colonoscopy). Three weeks before admission she completed a one-week triple therapy (pantoprazole 20 mg *bid*, clarithromycin 500 mg *bid* and amoxicillin 1 g *bid*) for *H. pylori* infection. On physical examination, she looked unhealthy, and her abdomen was mildly tender, with no masses. Temperature and vital signs were normal. Laboratory investigations revealed leukocytosis (14800/mm^3) with neutrophilia, high C-reactive protein (11.5 mg/dL), and low levels of serum albumin (2.4 mg/dL), sodium (133 mEq/L), and potassium (2.7 mEq/L). Two days before admission, stool samples examination excluded enteric bacterial pathogens (*Shighella*, *Salmonella*, *Yersinia* spp.) as well as *C. difficile* toxins. Without any prior preparation, the patient underwent a limited colonoscopy, which showed diffusely scattered off-white pseudomembranes attached to the hyperemic underlying mucosa and multiple diverticula (Figure 1). Repeated stool sample examination was positive for *C. difficile* toxins A and B. Treatment with oral metronidazole 500 mg every 8 h was initiated, replaced 72 h later with oral vancomycin 125 mg every 6 h due to unfavorable response. After a 10-d treatment with vancomycin, the patient had a complete resolution of the symptoms and was discharged from hospital with negative results for *C. difficile* toxins and one stool per day. During a four-month follow-up, patient remained asymptomatic.

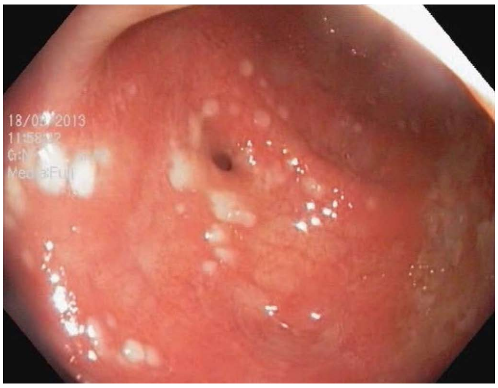
Figure 1 Pre-treatment endoscopic examination. Colonoscopy revealed scattered off-white pseudomembranes, some of them around a diverticulum.

The second case concerns a 71-year-old woman who was admitted with profuse watery diarrhea (up to 10 stools daily) and abdominal pain. Her prior medical history was unremarkable. Symptoms occurred 5 d after a one-week triple therapy (pantoprazole 20 mg *bid*, clarithromycin 500 mg *bid* and amoxicillin 1 g *bid*) for *H. pylori* eradication. Physical examination was normal, except for signs of dehydration. Microbiological examination of stools was negative for *Salmonella*, *Shigella* and *Yersinia* spp., and did not reveal any parasites. The patient had leukocytosis (12400/mm^3), hypokalemia (2.8 mEq/L), and mild inflammatory syndrome. Sigmoidoscopy showed typical signs of pseudomembranous colitis, while a stool test for *C. difficile* toxins A and B proved positive. The patient received 10-d treatment with oral vancomicyn 125 mg every 6 h, followed by prompt improvement in symptoms and negative test for *C. difficile* toxins. No relapse occurred during an 11-mo follow-up.

DISCUSSION

Eradication therapy for *H. pylori* provides enormous benefits and has proved to be both effective and safe. Except from the rare and mild side-effects, eradication therapy is generally well-tolerated. Severe adverse effects such as pseudomembranous colitis following eradication therapy have very rarely been reported[6-13] which is quite surprising, taken into account the immense number of subjects treated worldwide. It is difficult to find a clear explanation why are so rare cases of pseudomembranous colitis after eradication therapy reported in the literature, but some hypotheses were put forward: (1) the use of metronidazole, an efficient drug against *C. difficile*; however, several cases of pseudomembranous colitis, as published, occurred after a regimen containing metronidazole[8-10]; (2) the short duration of the therapy; (3) almost all treatments are carried out in outpatients (hospitalization is a risk factor for *C. difficile* infection); and (4) many cases with mild clinical disease were most likely not diagnosed, either because the patients did not consult a physician or the physician did not suspect the development of *C. difficile* infection[10].

Over the last decade, *C. difficile* infection rate has increased dramatically worldwide both in incidence and severity[14,15]. In addition to broad-spectrum antibiotic therapy, there have been identified many other potential risk factors for *C. difficile* infection (advanced age, female gender, comorbidities, admission to ICU, long hospital stay, immunosuppressive therapy, and PPI use)[16-21]. Several studies and recent meta-analyses have shown that PPI therapy is associated with increased risk of *C. difficile* infection[20-27], and United States Food and Drug Administration even issued a safety announcement to inform the public about this possible risk[28]. Newly published studies have found that *C. difficile* infection can occur outside the above mentioned well-known risk groups, in the absence of any hospitalization and even in young patients with no comorbidities[29].

All the components of the triple eradication therapy for *H. pylori* (PPI and two antibiotics: clarithromycin and amoxicillin or metronidazole) are potential risk factors for *C. difficile* infection. The most responsible for development of pseudomembranous colitis seems to be clarithromycin, used in both our cases and in most of the published reports[6,7,10,13].

The spectrum of *C. difficile* infection is wide, ranging from mild, self-limiting diarrhea to fulminant pseudomembranous colitis which is associated with significant morbidity and mortality. Most patients with *C. difficile* infection have a mild-to-moderate disease, but some may develop severe forms of disease such as pseudomembranous colitis, or even complicated by toxic megacolon[7]. Among variables used to define severe disease, the most important are the presence of pseudomembranes at endoscopy and age over 65 years. Both our cases met the criteria for a severe form of disease, and were treated with vancomycin according to current guidelines recommendations[30].

Our cases, in addition to the ones published, demonstrate that pseudomembranous colitis can occur after a usually well-tolerated triple therapy for *H. pylori* eradication. Despite this very rare complication, it should be underlined that *H. pylori* eradication therapy provides huge benefits and remains effective and generally safe. However, *C.difficile* infection may occur with an eradication therapy for *H. pylori* consisting of two antibiotics and a PPI. Most likely, *C. difficile* infection cases following *H. pylori* eradication therapy are not as rare as reported by literature, considering that a significant proportion of mild form of disease does not come to physician's attention and thus may remain undiagnosed[31]. Clinicians should be aware of such complication when prescribing triple therapy for *H. pylori* eradication, and should inform the patients that they may have diarrhea during or after treatment, and therefore should seek medical advice.

In conclusion, pseudomembranous colitis should be suspected in any patient with watery diarrhea during or after triple therapy for *H. pylori* eradication. Awareness of such complication is particularly important in the actual context when both duration and indications for *H. pylori* eradication therapy have been extended.

REFERENCES

1 **Hunt RH**, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, van der Merwe S, Vaz Coelho LG, Fock M, Fedail S, Cohen H, Malfertheiner P, Vakil N, Hamid S, Goh KL, Wong BC, Krabshuis J, Le Mair A. Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. *J Gastrointestin Liver Dis* 2011; **20**: 299-304 [PMID: 21961099 DOI: 10.1097/MCG.0b013e31820fb8f6]

2 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

3 Current European concepts in the management of Helicobacter pylori infection. The Maastricht Consensus Report. European Helicobacter Pylori Study Group. *Gut* 1997; **41**: 8-13 [PMID: 9274464]

4 **Megraud F**. Helicobacter pylori and antibiotic resistance. *Gut* 2007; **56**: 1502 [PMID: 17938430 DOI: 10.1136/gut.2007.132514]

5 **Graham DY**, Fischbach L. Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut* 2010; **59**: 1143-1153 [PMID: 20525969 DOI: 10.1136/gut.2009.192757]

6 **Teare JP**, Booth JC, Brown JL, Martin J, Thomas HC. Pseudomembranous colitis following clarithromycin therapy. *Eur J Gastroenterol Hepatol* 1995; **7**: 275-277 [PMID: 7743311]

7 **Schweigart U**, Franck H, Schepp W, Lehn N, Becker K, Classen M. Toxic megacolon after Helicobacter pylori eradication therapy. *Internist* (Berl) 1997; **38**: 352-354 [PMID: 9213574]

8 **Archimandritis A**, Souyioultzis S, Katsorida M, Tzivras M. Clostridium difficile colitis associated with a 'triple' regimen, containing clarithromycin and metronidazole, to eradicate Helicobacter pylori. *J Intern Med* 1998; **243**: 251-253 [PMID: 9627163 DOI: 10.1046/j.1365-2796.1998.00272.x]

9 **Nawaz A**, Mohammed I, Ahsan K, Karakurum A, Hadjiyane C, Pellecchia C. Clostridium difficile colitis associated with treatment of Helicobacter pylori infection. *Am J Gastroenterol* 1998; **93**: 1175-1176 [PMID: 9672359]

10 **Harsch IA**, Hahn EG, Konturek PC. Pseudomembranous colitis after eradication of Helicobacter pylori infection with a triple therapy. *Med Sci Monit* 2001; **7**: 751-754 [PMID: 11433206]

11 **Lau CF**, Hui PK, Fung TT, Tung SY, Wong AM, Loo CK, Lam KM. Pseudomembranous colitis without diarrhoea following Helicobacter pylori eradication therapy. *Hosp Med* 2001; **62**: 431-433 [PMID: 11480134]

12 **Rai R**, Rai S. Pseudomembranous colitis requiring surgical intervention following triple therapy for Helicobacter pylori eradication. *ANZ J Surg* 2002; **72**: 917-919 [PMID: 12485235 DOI: 10.1046/j.1445-2197.2002.02593.x]

13 **Kubo N**, Kochi S, Ariyama I, Murata M, Furusyo N, Hayashi J. Pseudomembranous colitis after Helicobacter pylori eradication therapy. *Kansenshogaku Zasshi* 2006; **80**: 51-55 [PMID: 16519125]

14 **Ananthakrishnan AN**. Clostridium difficile infection: epidemiology, risk factors and management. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 17-26 [PMID: 21119612 DOI: 10.1038/

nrgastro.2010.190]

15 **O'Donoghue C**, Kyne L. Update on Clostridium difficile infection. *Curr Opin Gastroenterol* 2011; **27**: 38-47 [PMID: 21099432 DOI: 10.1097/MOG.0b013e3283411634]

16 **Khanna S**, Pardi DS. The growing incidence and severity of Clostridium difficile infection in inpatient and outpatient settings. *Expert Rev Gastroenterol Hepatol* 2010; **4**: 409-416 [PMID: 20678014 DOI: 10.1586/egh.10.48]

17 **Morrison RH**, Hall NS, Said M, Rice T, Groff H, Brodine SK, Slymen D, Lederman ER. Risk factors associated with complications and mortality in patients with Clostridium difficile infection. *Clin Infect Dis* 2011; **53**: 1173-1178 [PMID: 21976459 DOI: 10.1093/cid/cir668]

18 **Dubberke ER**, Reske KA, Olsen MA, McMullen KM, Mayfield JL, McDonald LC, Fraser VJ. Evaluation of Clostridium difficile-associated disease pressure as a risk factor for C difficile-associated disease. *Arch Intern Med* 2007; **167**: 1092-1097 [PMID: 17533213 DOI: 10.1086/52582]

19 **Kuntz JL**, Chrischilles EA, Pendergast JF, Herwaldt LA, Polgreen PM. Incidence of and risk factors for community-associated Clostridium difficile infection: a nested case-control study. *BMC Infect Dis* 2011; **11**: 194 [PMID: 21762504 DOI: 10.1186/1471-2334-11-194]

20 **Al-Tureihi FI**, Hassoun A, Wolf-Klein G, Isenberg H. Albumin, length of stay, and proton pump inhibitors: key factors in Clostridium difficile-associated disease in nursing home patients. *J Am Med Dir Assoc* 2005; **6**: 105-108 [PMID: 15871884 DOI: 10.1016/j.jamda.2005.01.003]

21 **Dalton BR**, Lye-Maccannell T, Henderson EA, Maccannell DR, Louie TJ. Proton pump inhibitors increase significantly the risk of Clostridium difficile infection in a low-endemicity, non-outbreak hospital setting. *Aliment Pharmacol Ther* 2009; **29**: 626-634 [PMID: 19183143 DOI: 1111/j.1365-2036.2008.03924.x]

22 **Aseeri M**, Schroeder T, Kramer J, Zackula R. Gastric acid suppression by proton pump inhibitors as a risk factor for clostridium difficile-associated diarrhea in hospitalized patients. *Am J Gastroenterol* 2008; **103**: 2308-2313 [PMID: 18702653 DOI: 10.1111/j.1572-0241.2008.01975.x]

23 **Linney S**, Fernandes T, Einarson T, Sengar A, Walker JH, Mills A. Association Between Use of Proton Pump Inhibitors and a Clostridium difficile-Associated Disease Outbreak: Case-Control Study. *Can J Hosp Pharm* 2010; **63**: 31-37 [PMID: 22478951]

24 **Kwok CS**, Arthur AK, Anibueze CI, Singh S, Cavallazzi R, Loke YK. Risk of Clostridium difficile infection with acid suppressing drugs and antibiotics: meta-analysis. *Am J Gastroenterol* 2012; **107**: 1011-1019 [PMID: 22525304 DOI: 10.1038/ajg.2012.108]

25 **Deshpande A**, Pant C, Pasupuleti V, Rolston DD, Jain A, Deshpande N, Thota P, Sferra TJ, Hernandez AV. Association between proton pump inhibitor therapy and Clostridium difficile infection in a meta-analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 225-233 [PMID: 22019794 DOI: 10.1016/j.cgh.2011.09.030]

26 **Tleyjeh IM**, Bin Abdulhak AA, Riaz M, Alasmari FA, Garbati MA, AlGhamdi M, Khan AR, Al Tannir M, Erwin PJ, Ibrahim T, Allehibi A, Baddour LM, Sutton AJ. Association between proton pump inhibitor therapy and clostridium difficile infection: a contemporary systematic review and meta-analysis. *PLoS One* 2012; **7**: e50836 [PMID: 23236397 DOI: 10.1371/journal.pone.0050836]

27 **Howell MD**, Novack V, Grgurich P, Soulliard D, Novack L, Pencina M, Talmor D. Iatrogenic gastric acid suppression and the risk of nosocomial Clostridium difficile infection. *Arch Intern Med* 2010; **170**: 784-790 [PMID: 20458086 DOI: 10.1001/archinternmed.2010.89]

28 **US Food and Drug Administration**. Clostridium difficile-associated diarrhea can be associated with stomach acid drugs known as proton pump inhibitors (PPIs) 2012. RDA Drug Safety Communication, Rockville, MD, USA, accessed March 2012. Available from: URL: http://www.fda.gov/Drugs/DrugSafety/ucm 290510.htm

29 **Khanna S**, Pardi DS, Aronson SL, Kammer PP, Orenstein R, St Sauver JL, Harmsen WS, Zinsmeister AR. The epidemiology of community-acquired Clostridium difficile infection: a population-based study. *Am J Gastroenterol* 2012; **107**: 89-95 [PMID: 22108454 DOI: 10.1038/ajg.2011.398]

30 **Surawicz CM**, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am J Gastroenterol* 2013; **108**: 478-98; quiz 499 [PMID: 23439232 DOI: 10.1038/ajg.2013.4]

31 **Alcalá L**, Martín A, Marín M, Sánchez-Somolinos M, Catalán P, Peláez T, Bouza E; Spanish Clostridium difficile Study Group. The undiagnosed cases of Clostridium difficile infection in a whole nation: where is the problem? *Clin Microbiol Infect* 2012; **18**: E204-E213 [PMID: 22563775 DOI: 10.1111/j.1469-0691.2012.03883]

P- Reviewers: VetvickaV, Wasko-Czopnik D
S- Editor: Zhai HH **L- Editor**: A **E- Editor**: Ma S

World Journal of
Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7480

World J Gastroenterol 2013 November 14; 19(42): 7480-7486
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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CASE REPORT

Successful treatment of multiple hepatocellular adenomas with percutaneous radiofrequency ablation

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Received: July 25, 2013 **Revised:** September 5, 2013
Accepted: September 15, 2013
Published online: November 14, 2013

Abstract

Hepatocellular adenoma (HCA) is one of the important complications of glycogen storage disease type Ⅰa (GSD-Ⅰa) because it can be transformed into hepatocellular carcinoma. Although surgical resection is a standard treatment of choice for solitary HCA, multiple HCAs in GSD-Ⅰa patients present as therapeutic challenges for curative treatment. Therefore, treatment strategy according to malignant potential is important in management of HCAs in GSD-Ⅰa. The authors present a case of histologically proven multiple HCAs without β-catenin mutations occurred in a GSD-Ⅰa patient treated successfully with percutaneous radiofrequency ablation as a minimally invasive therapy.

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Key words: Glycogen storage disease; Hepatocellular adenoma; Radiofrequency ablation; β-catenin activation; Glycogen storage disease

Core tip: Risk stratification by pathological examination is an important step in deciding therapeutic options of multiple hepatocellular adenoma in glycogen storage disease type Ⅰa patients.

Ahn SY, Park SY, Kweon YO, Tak WY, Bae HI, Cho SH. Successful treatment of multiple hepatocellular adenomas with percutaneous radiofrequency ablation. *World J Gastroenterol* 2013; 19(42): 7480-7486 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7480.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7480

INTRODUCTION

Glycogen storage disease type Ⅰa (GSD-Ⅰa) is an inherited disorder of carbohydrate metabolism caused by glucose-6-phosphatase deficiency in the liver, kidneys and intestinal mucosa[1]. Liver diseases associated with GSD-Ⅰa include hepatocellular adenoma (HCA) which can lead to considerable morbidity and mortality associated with malignant transformation and intratumoral hemorrhage[2]. While HCA associated with oral contraceptives or exogenous androgen is relatively large, single and encapsulated HCAs in GSD-Ⅰa tend to be small, multiple and non-capsulated[3]. Furthermore, HCAs in GSD-Ⅰa tend to transform into hepatocellular carcinoma (HCC) more frequently than sporadic HCAs[3]. Rates of transformation into HCC have been reported approximately as being up to 10%[4]. Recently, sporadic HCAs have been classified into four subgroups; hepatocytic nuclear factor 1 α mutated HCAs, β-catenin-activated HCAs, inflammatory HCA, and unclassified HCA according to molecular markers and immunohistochemistry[5]. Because β-catenin activation is known as a risk factor for malignant transformation, subgroup classification of HCAs has been suggested as an important step in management of HCAs[6,7]. Although several strategies for management of multiple HCAs in GSD- I a are suggested in literature, the best treatment is still controversial[8]. Surgical resection has been a standard treatment for solitary HCA with high risk of malignancy. However, the role of surgical resection is limited in multiple HCAs in GSD- I a. Liver transplantation could be another treatment option for management of multiple HCAs as well as correction of most metabolic derangement in GSD- I a patients[9]. Other options such as ethanol injection and transarterial embolization have been suggested as new and less invasive treatments[10-12]. The authors present a case of multiple HCAs without β-catenin mutations in a patient with GSD- I a which were successfully treated by ultrasonography guided percutaneous radiofrequency ablation (RFA).

CASE REPORT

A 24-year-old male patient was referred for multiple liver masses. He was diagnosed as GSD- I a 10 years ago. He had a family history of two brothers who died of HCC at the age of 22 and 26 years, respectively. His mother died from an undetermined cause in her twenties. After diagnosis, he had never received dietary therapy or other medical treatments. He was undersized with a height of 166 cm and weight of 51 kg. The laboratory findings revealed hyperlipidemia and hyperuricemia after a 12 h fast (Table 1). Serologic tests for viral hepatitis and autoimmune liver disease were all negative. Serum levels of α-fetoprotein and prothrombin induced by vitamin K deficiency or antagonist-Ⅱ were 2.4 ng/mL and 35 mAU/mL. Mutation analysis of the glucose-6-phosphatase gene revealed compound heterozygosity for p.Leu216X (c.648G > T) and p.Gly222Arg (c.664G > A) in exon 5. On subsequent gadolinium ethoxybenzyl diethylene triamine pentaacetic acid (Gd-EOB-DTPA; Primovist, Bayer Schering Pharma, Berlin, Germany) enhanced magnetic resonance imaging (MRI), four arterial-phase enhancing nodules were noted in both lobes of liver and these nodules showed clear defects on 20 min hepatobiliary phase (Figure 1). Three nodules revealed the same features with intense homogeneous arterial enhancement, nearly iso-signal intensity with capsular enhancement on 3 min delayed phase, and intermediate high signal intensity on axial T2-weighted image (T2WI). There was no evidence of fat component or internal hemorrhage, or the Atoll sign (Figure 1A-C). One nodule showed heterogeneous signal intensity on 3 min delayed phase, axial T2WI, and 20 min hepatobiliary phase (Figure 1D). These features were consistent with multiple hepatic adenomas associated with GSD- I a. We performed percutaneous liver biopsies on each nodule to identify HCA subtype. Each biopsy specimen underwent standard histopathological examination and immunohistochemistry for β-catenin, glutamine synthetase and amyloid A (Figure 2). Immunohistochemistry confirmed all the adenomas as inflammatory type HCA without malignant transformation. As all the nodules were small without malignant cells and β-catenin activation, we performed ultrasonography guided RFA as a less invasive therapeutic option. RFA was successfully performed under local anesthesia using a cool-tip electrode with a 3 cm exposed tip (ACT2030, Covidien, Mansfield, MA, United States). Multiple overlapping ablation methods were applied for each adenoma. There was no procedure-related complication after RFA. Three months follow-up computed tomogram scans revealed gradual reduction of the RFA zone without any residual tumor (Figure 3).

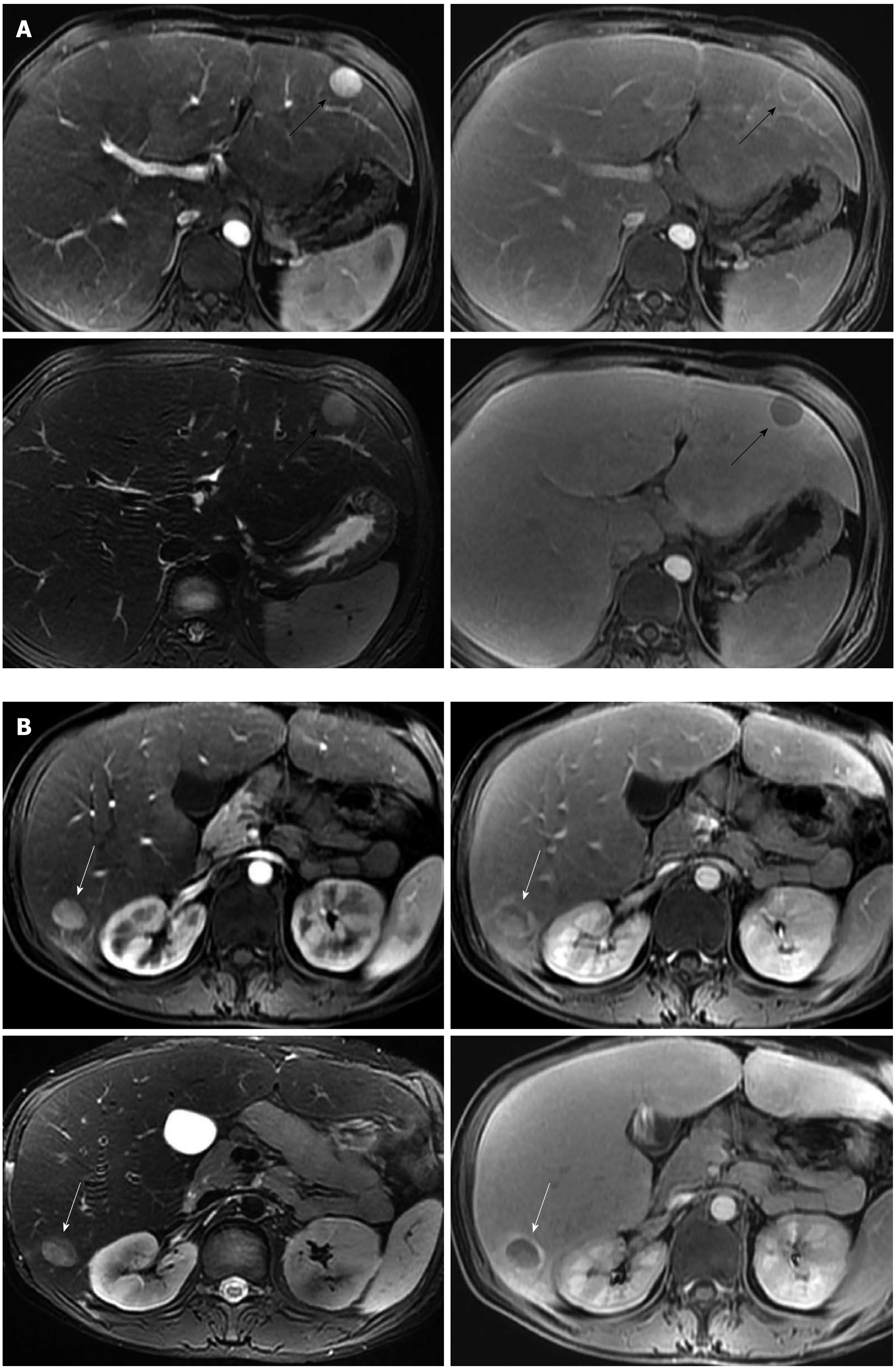
Table 1 Laboratory findings on admission

Blood chemistry	Value
Total bilirubin	0.42 mg/dL
ALP	218 U/L
AST	87 U/L
ALT	37 U/L
LDH	275 U/L
γ-GTP	152 U/L
Total protein	8.9 g/dL
Albumin	4.8 g/dL
Total cholesterol	380 mg/dL
TG	1688 mg/dL
Uric acid	9.4 mg/dl
BUN	8.5 mg/dL
Creatinine	1.15 mg/dL
Glucose	93 mg/dL
Lactic acid	3.5 mmol/L
Hemoglobin A1c	5.0%
Hematology	
Hemoglobin	11.3 g/dL
White blood cell	4.14 × 10^3/μL
Platelet	269 × 10^3/μL
Serology	
AFP	2.2 ng/mL
PIVKA-Ⅱ	35 mAU/mL
Hepatitis B surface antigen	Negative
Anti HBs Ab	Negative
Hepatitis B core antibody	Negative
Anti HCV Ab	Negative
Hemostasis	
Prothrombin time	11.2 s

ALP: Alkaline phosphatase; AST: Aspartate transaminase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase; γ-GTP: Gammaglutamyl transpeptidase; TG: Triglyceride; BUN: Blood urea nitrogen; AFP: α-fetoprotein; PIVKA-Ⅱ: Prothrombin induced by vitamin K deficiency or antagonist-Ⅱ.

DISCUSSION

Management of HCAs in patients with GSD- I a is not well established because of the rarity of this disease. As patients with GSD- I a survive longer with intensive dietary therapy, they have more chances to develop HCAs in their expanded life span[13]. Although there is a case report that HCA in GSD- I a regressed after strict dietary therapy, maintenance of normoglycemia under intensive dietary therapy is insufficient to regress HCAs or reverse malignant transformation because patients with multiple HCAs sometimes showed sudden progression and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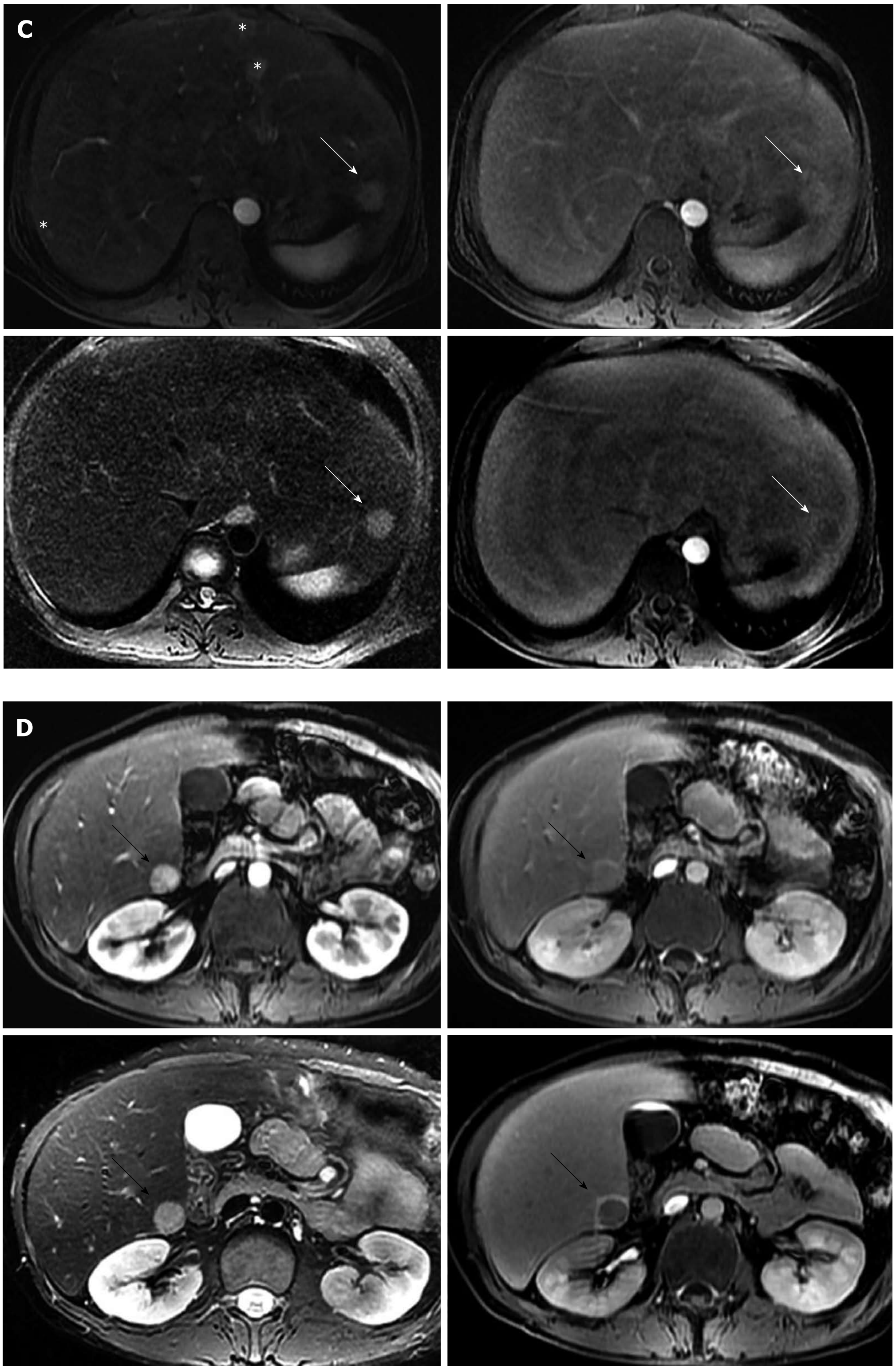

Figure 1 Hepatic adenomas associated with glycogen storage disease type Ⅰ a. A: Gd-EOB-DTPA enhanced axial T1-weighted image on arterial phase (left upper) reveals approximately 2 cm intense enhancing mass in the segment 3 of liver (black arrows). The mass shows nearly iso-signal intensity with capsular enhancement on 3 min delayed phase (right upper), intermediate high signal intensity on axial T2-weighted image (left lower), and clear defect on 20 min hepatobiliary phase (right lower); B: Approximately 1.8 cm sized intense enhancing mass on arterial phase (left upper) is noted in the segment 6 of liver. This mass shows suspicious focal eccentric wash-out enhancement (white arrows) on 3 min delayed phase (right upper). This portion reveals relatively low signal intensity on axial T2-weighted image (left lower) and 20 min hepatobiliary phase (right lower), compared to other tumor area. Surrounding rim enhancement in the tumor is seen on 20 min hepatobiliary phase; C: Approximately 1.8 cm arterial enhancing mass (white arrows) is seen in the segment 2 of liver. The mass reveals slightly high signal intensity on 3 min delayed phase (right upper), intermediate high signal intensity on axial T2-weighted image (left lower), and fuzzy defect on 20 min hepatobiliary phase (right lower). Multifocal arterioportal shunts (asterisks) are noted in the segments 3 and 7 of liver; D: Approximately 1.8 cm sized intense enhancing mass (black arrows) on arterial phase (left upper) is noted in the segment 6 of liver. This mass shows slightly high signal intensity with capsular enhancement on 3min delayed phase (right upper), and intermediate high signal intensity on axial T2-weighted image (left lower). On 20 min hepatobiliary phase (right lower), the tumor reveals clear defect with surrounding rim enhancement. Gd-EOB-DTPA: Gadolinium ethoxybenzyl diethylene triamine pentaacetic acid.

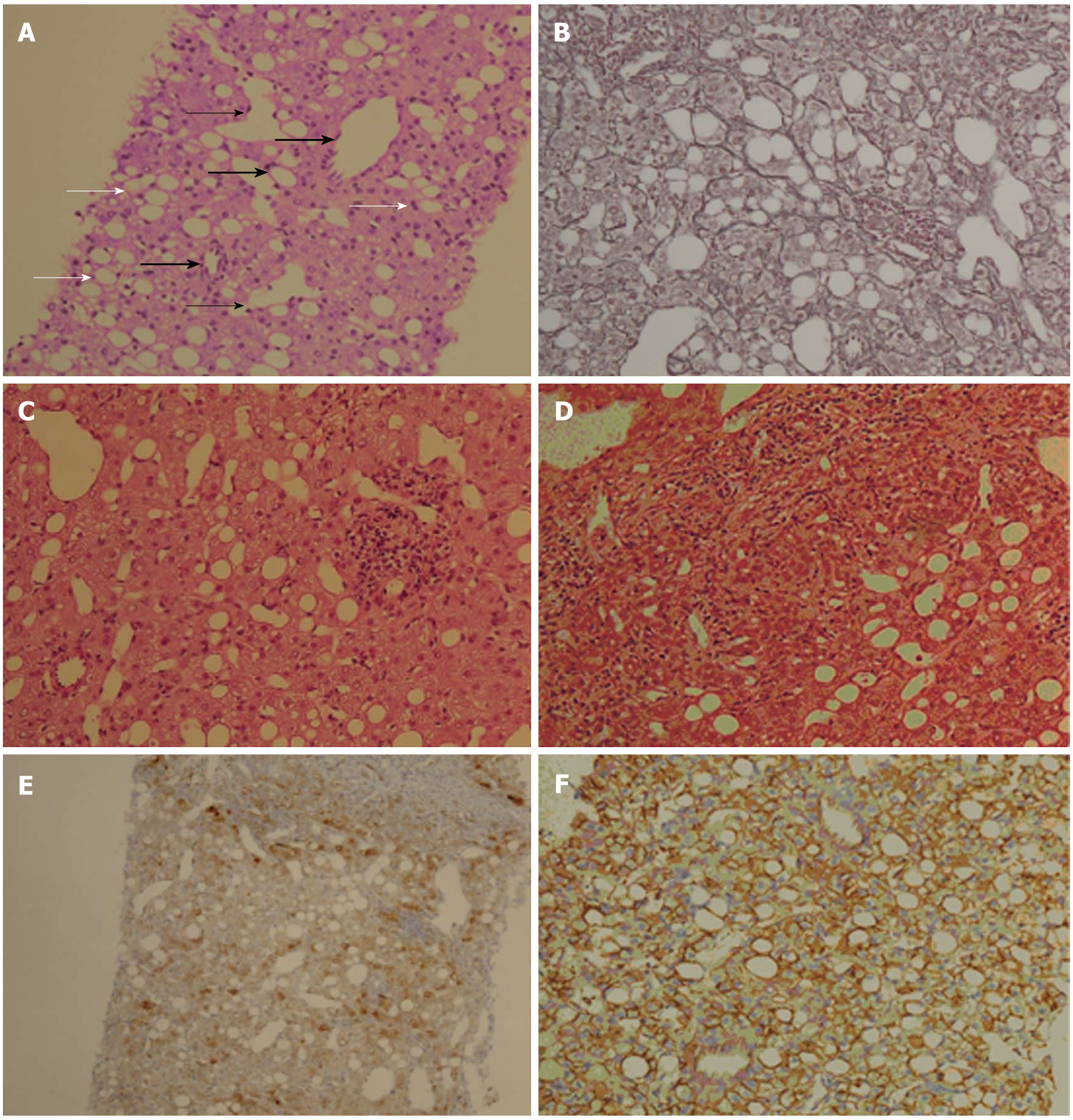

Figure 2 The histological features are consistent with the inflammatory type of hepatocellular adenoma. A: A hepatocellular adenoma in GSD-Ia has several unpaired arteries (thick black arrows), sinusoidal dilation (thin black arrows), and steatosis (white arrows), but the atypia of hepatocytes are low without a nuclear pleomorphism; B: A reticulin staining does not show thick hepatic cords; C: The lymphocytic infiltrates are found in a hepatic lobule; D: The lymphocytes infiltrate in the area surrounded a portal and a periportal tracts; E: Staining for glutamine synthetase is diffuse cytoplasmic with focal nuclear expression; F: β-catenin staining does not show abnormal expression. GSD-Ia: Glycogen storage disease type Ⅰa.

metastatic spread[3,14-17]. As HCAs in GSD-Ⅰa tend to be multiple, involving both lobes of liver, the ultimate treatment of HCAs in GSD-Ⅰa is liver transplantation for management of HCAs and to reverse metabolic derangement[9,18]. However, liver transplantation is rarely performed due to uncertainties on timing of transplantation, limited organ availability and combined renal dysfunction with immunosuppression[13]. Surgical resection of HCAs is suggested as an effective intermediate therapy for prevention of HCC until liver transplantation[13]. However, liver resection in GDS-Ⅰa patients showed greater morbidity including intra-abdominal abscess, multi-organ failure, unstable blood glucose control and hemorrhage.

Recently, molecular characterizations of HCAs demonstrated that frequent β-catenin mutations are more frequently observed in HCAs in GSD-Ⅰa and related to increased risk of malignant transformation like sporadic HCAs[19]. To assess the risk of malignant transformation, we biopsied each HCA for analysis of subtype of each tumor by immunohistochemistry. All the HCAs were small and inflammatory type with low risk of malignant transformation. There are reports of treatment of HCAs in GSD-Ⅰa by transarterial embolization and ethanol injection as a minimal invasive procedure[11,12]. As RFA can offer complete necrosis of target lesions compared to transarterial embolization and ethanol injection, we treated HCAs by percutaneous RFA successfully. The present case shows the potential role of RFA in management of multiple HCAs in GSD-Ⅰa patients along with risk stratification by pathological examination.

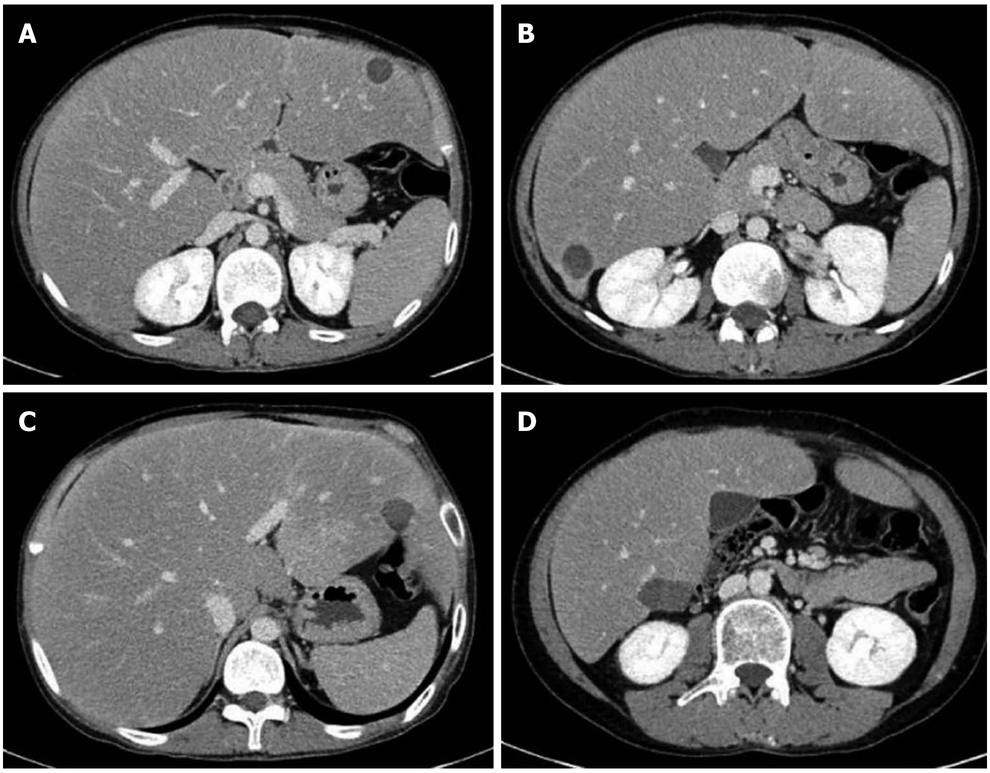

Figure 3 Computed tomogram follow-up after 3 mo showed gradual reduction of radiofrequency ablation zone. A: Post-RFA follow-up CT revealed gradual contraction of ablated lesion in segment 3 to 1 cm; B: Complete ablation of enhancing lesion in segment 6 was observed in Post-RFA follow-up CT; C: Post-RFA follow-up CT showed complete ablation of 1.8 cm sized tumor in segment 2; D: Post-RFA CT revealed no residual lesion without adjacent organ damage. CT: Computed tomogram; RFA: Radiofrequency ablation.

REFERENCES

1 **Franco LM**, Krishnamurthy V, Bali D, Weinstein DA, Arn P, Clary B, Boney A, Sullivan J, Frush DP, Chen YT, Kishnani PS. Hepatocellular carcinoma in glycogen storage disease type Ia: a case series. *J Inherit Metab Dis* 2005; **28**: 153-162 [PMID: 15877204 DOI: 10.1007/s10545-005-7500-2]

2 **Wang DQ**, Fiske LM, Carreras CT, Weinstein DA. Natural history of hepatocellular adenoma formation in glycogen storage disease type I. *J Pediatr* 2011; **159**: 442-446 [PMID: 21481415 DOI: 10.1016/j.jpeds.2011.02.031]

3 **Sakellariou S**, Al-Hussaini H, Scalori A, Samyn M, Heaton N, Portmann B, Tobal K, Quaglia A. Hepatocellular adenoma in glycogen storage disorder type I: a clinicopathological and molecular study. *Histopathology* 2012; **60**: E58-E65 [PMID: 22372484 DOI: 10.1111/j.1365-2559.2011.04153.x]

4 **Lee PJ**. Glycogen storage disease type I: pathophysiology of liver adenomas. *Eur J Pediatr* 2002; **161** Suppl 1: S46-S49 [PMID: 12373570 DOI: 10.1007/s00431-002-1002-0]

5 **Bioulac-Sage P**, Rebouissou S, Thomas C, Blanc JF, Saric J, Sa Cunha A, Rullier A, Cubel G, Couchy G, Imbeaud S, Balabaud C, Zucman-Rossi J. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. *Hepatology* 2007; **46**: 740-748 [PMID: 17663417 DOI: 10.1002/hep.21743]

6 **Bioulac-Sage P**, Laumonier H, Couchy G, Le Bail B, Sa Cunha A, Rullier A, Laurent C, Blanc JF, Cubel G, Trillaud H, Zucman-Rossi J, Balabaud C, Saric J. Hepatocellular adenoma management and phenotypic classification: the Bordeaux experience. *Hepatology* 2009; **50**: 481-489 [PMID: 19585623 DOI: 10.1002/hep.22995]

7 **Nault JC**, Bioulac-Sage P, Zucman-Rossi J. Hepatocellular benign tumors-from molecular classification to personalized clinical care. *Gastroenterology* 2013; **144**: 888-902 [PMID: 23485860 DOI: 10.1053/j.gastro.2013.02.032]

8 **van Aalten SM**, Witjes CD, de Man RA, Ijzermans JN, Terkivatan T. Can a decision-making model be justified in the management of hepatocellular adenoma? *Liver Int* 2012; **32**: 28-37 [PMID: 22098685 DOI: 10.1111/j.1478-3231.2011.02667.x]

9 **Reddy SK**, Austin SL, Spencer-Manzon M, Koeberl DD, Clary BM, Desai DM, Smith AD, Kishnani PS. Liver transplantation for glycogen storage disease type Ia. *J Hepatol* 2009; **51**: 483-490 [PMID: 19596478 DOI: 10.1016/j.jhep.2009.05.026]

10 **Rocourt DV**, Shiels WE, Hammond S, Besner GE. Contemporary management of benign hepatic adenoma using percutaneous radiofrequency ablation. *J Pediatr Surg* 2006; **41**: 1149-1152 [PMID: 16769351 DOI: 10.1016/j.jpedsurg.2006.01.064]

11 **Karkar AM**, Tang LH, Kashikar ND, Gonen M, Solomon SB, Dematteo RP, D' Angelica MI, Correa-Gallego C, Jarnagin WR, Fong Y, Getrajdman GI, Allen P, Kingham TP. Management of hepatocellular adenoma: comparison of resection, embolization and observation. *HPB (Oxford)* 2013; **15**: 235-243 [PMID: 23374365 DOI: 10.1111/j.1477-2574.2012.00584.x]

12 **Yoshikawa M**, Fukui K, Kuriyama S, Tsujimoto T, Nakatani Y, Toyokawa Y, Kurematsu Y, Awata J, Shiroi A, Fukui H, Tsutsumi M. Hepatic adenomas treated with percutaneous ethanol injection in a patient with glycogen storage disease type Ia. *J Gastroenterol* 2001; **36**: 52-61 [PMID: 11211213]

13 **Reddy SK**, Kishnani PS, Sullivan JA, Koeberl DD, Desai DM, Skinner MA, Rice HE, Clary BM. Resection of hepatocellular adenoma in patients with glycogen storage disease type Ia. *J Hepatol* 2007; **47**: 658-663 [PMID: 17637480 DOI: 10.1016/j.jhep.2007.05.012]

14 **Rake JP**, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GP. Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of the European Study

on Glycogen Storage Disease Type I (ESGSD I). *Eur J Pediatr* 2002; **161** Suppl 1: S20-S34 [PMID: 12373567 DOI: 10.1007/s00431-002-0999-4]

15 **Yiu WH**, Pan CJ, Mead PA, Starost MF, Mansfield BC, Chou JY. Normoglycemia alone is insufficient to prevent long-term complications of hepatocellular adenoma in glycogen storage disease type Ib mice. *J Hepatol* 2009; **51**: 909-917 [PMID: 19376605 DOI: 10.1016/j.jhep.2008.11.026]

16 **Zangeneh F**, Limbeck GA, Brown BI, Emch JR, Arcasoy MM, Goldenberg VE, Kelley VC. Hepatorenal glycogenosis (type I glycogenosis) and carcinoma of the liver. *J Pediatr* 1969; **74**: 73-83 [PMID: 4302545]

17 **Mikuriya Y**, Oshita A, Tashiro H, Amano H, Kobayashi T, Arihiro K, Ohdan H. Hepatocellular carcinoma and focal nodular hyperplasia of the liver in a glycogen storage disease patient. *World J Hepatol* 2012; **4**: 191-195 [PMID: 22761970 DOI: 10.4254/wjh.v4.i6.191]

18 **Belingheri M**, Ghio L, Sala A, Menni F, Trespidi L, Ferraresso M, Berardinelli L, Rossi G, Edefonti A, Parini R. Combined liver-kidney transplantation in glycogen storage disease Ia: a case beyond the guidelines. *Liver Transpl* 2007; **13**: 762-764 [PMID: 17457869 DOI: 10.1002/lt.21147]

19 **Calderaro J**, Labrune P, Morcrette G, Rebouissou S, Franco D, Prévot S, Quaglia A, Bedossa P, Libbrecht L, Terracciano L, Smit GP, Bioulac-Sage P, Zucman-Rossi J. Molecular characterization of hepatocellular adenomas developed in patients with glycogen storage disease type I. *J Hepatol* 2013; **58**: 350-357 [PMID: 23046672 DOI: 10.1016/j.jhep.2012.09.030]

P- Reviewer: Bannasch P **S- Editor**: Ma YJ
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World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7487

World J Gastroenterol 2013 November 14; 19(42): 7487-7488
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

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LETTERS TO THE EDITOR

Rifaximin and Crohn's disease

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Received: July 3, 2013 **Revised:** September 2, 2013
Accepted: September 15, 2013
Published online: November 14, 2013

Abstract

In a recent article, Longman and Swaminath analyzed our paper on the use of rifaximin in patients with moderately active Crohn's disease (CD). Here we report some considerations concerning their article. The exploratory *post-hoc* subgroup analysis showed that early-stage disease and, differently from that written by Longman and Swaminath, also colonic involvement seemed to be associated with a significant higher efficacy of rifaximin-EIR 800 mg twice daily. Early-stage disease is generally considered as the more easily treatable phase of CD, and the better response to rifaximin in Crohn's colitis is in accordance with the high concentration of bacteria in the colon. In addition, patients with C reactive protein level > 5 mg/L achieved remission more significantly than patients with normal values, thus suggesting that the symptoms were probably caused by inflammation instead of by non-inflammatory causes. We also analyze the role of rifaximin against gut bacteria and the clinical situations that could obtain the best results from antibiotics.

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Key words: Crohn's disease; Intestinal microbiota; Non-absorbable antibiotic; Rifaximin

Core tip: In this letter to the Editor we report some considerations concerning the article entitled "Microbial manipulation as primary therapy for Crohn's disease" written by Longman and Swaminath. In the article the authors analyzed our paper on the use of rifaximin as primary therapy in active Crohn's disease. The *post-hoc* analysis of our study showed that early-stage disease, colonic involvement and a C reactive protein level > 5 mg/L were associated with a significant higher efficacy of rifaximin. We also discuss the role of rifaximin against intestinal bacteria and the clinical situations to explore further in controlled studies with antibiotics.

Prantera C, Scribano ML. Rifaximin and Crohn's disease. *World J Gastroenterol* 2013; 19(42): 7487-7488 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i42/7487.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i42.7487

TO THE EDITOR

We would like to thank Doctor Longman and Swaminath from Columbia University for their interesting analysis of our article concerning the trial of rifamixin in Crohn's disease (CD)[1,2].

Their expert analysis entitled "Microbial manipulation as primary therapy for Crohn's disease" merits some considerations and one slight correction: (1) The *post-hoc* explorative subgroup analysis has certainly revealed that patients with an early disease (as defined as first diagnosis ≤ 3 years before enrollment in the study) are significantly more likely to achieve remission with rifaximin-EIR 800 mg twice a day compared to placebo, but, differently from what Longman and Swaminath have written, also colonic involvement, and a baseline C-reactive protein (CRP) level > 5 mg/L showed the same statistical significant superiority. The favorable effect of these three factors is biologically plausible. Elevated CRP values suggest that the symptoms, which increase the Crohn's disease activity index values, are probably caused by inflammation instead of by non-inflammatory causes such as irritable bowel, bile salt diarrhoea or previous surgery. Many previous studies with antibiotics have shown that Crohn' s colitis responds better to this therapy than does that in the small bowel location, and this is in accordance with the higher microbial content in the colon. Finally, early disease is more easily treatable because the lesions are mainly inflammatory and the structural damage has usually not yet appeared; (2) Rifaximin is a non-absorbable antibiotic and consequently it should not work on the bacteria attached to the mucosa. Rifaximin can work against bacteria present in the lumen but not against adherent *Escherichia coli* (AIEC), and this fact could be particularly important if AIEC is one of the causes of CD[3]. At most rifaximin can reduce the attachment of enteroaggregative *Escherichia coli* by biologically altering the host cell, but it should not able to counteract the bacteria already adherent to the mucosa[4]. A study which explores the efficacy of one antibiotic together with a microbial analysis in order to ascertain which bacteria are involved in the inflammatory process, cannot be limited to investigating the stools flora, given that the bacteria found in the stools probably do not represent the adherent bacteria and could not be responsible for the inflammation; (3) From our study about 50% of the patients maintained clinical remission 12 wk after stopping rifaximin. It is probable, however, that a permanent change of the microbiota does not occur; and (4) Doctors Longman and Swaminath ask how to select patients who could obtain the best benefit from antibiotics. Crohn's colitis and elevated values of acute phase reactants seem the better clinical situation to explore further in controlled studies. Given the possible role of adherent bacteria in causing CD or, at least, in being responsible for symptoms, another very promising situation should be explored: immediately after surgery, when all the diseased tracts have been removed, antimicrobial therapy could obstruct the adherence of bacteria to the mucosa reducing the recurrence of lesions.

REFERENCES

1 **Longman RS**, Swaminath A. Microbial manipulation as primary therapy for Crohn's disease. *World J Gastroenterol* 2013; **19**: 1513-1516 [PMID: 23539531 DOI: 10.3748/wjg.v19.i10.1513]

2 **Prantera C**, Lochs H, Grimaldi M, Danese S, Scribano ML, Gionchetti P; Retic Study Group (Rifaximin-Eir Treatment in Crohn's Disease). Rifaximin-extended intestinal release induces remission in patients with moderately active Crohn's disease. *Gastroenterology* 2012; **142**: 473-481 [PMID: 22155172 DOI: 10.1053/j.gastro.2011.11.032]

3 **Swidsinski A**, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002; **122**: 44-54 [PMID: 11781279 DOI: 10.1053/gast.2002.30294]

4 **Brown EL**, Xue Q, Jiang ZD, Xu Y, Dupont HL. Pretreatment of epithelial cells with rifaximin alters bacterial attachment and internalization profiles. *Antimicrob Agents Chemother* 2010; **54**: 388-396 [PMID: 19858255 DOI: 10.1128/AAC.00691-09]

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WJG World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
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World J Gastroenterol 2013 November 14; 19(42): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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Name of journal

World Journal of Gastroenterology

ISSN

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Launch date

October 1, 1995

Frequency

Weekly

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6

24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23243641.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
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ISSN 1007-9327
9 771007 932045 42

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